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Citation: *Appl. Phys. Lett.* **102**, 203706 (2013); doi: 10.1063/1.4807837

View online: <http://dx.doi.org/10.1063/1.4807837>

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Bio-inspired optofluidic lasers with luciferin

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(Received 8 March 2013; accepted 13 May 2013; published online 24 May 2013)

The authors demonstrate a bio-inspired optofluidic laser with luciferin, a class of light-emitting compounds synthesized by many different organisms, as the gain medium. The laser characteristics under various conditions such as solution pH value and luciferin concentration are investigated. The authors demonstrate an optofluidic fluorescence resonance energy transfer laser by using luciferin and Rhodamine 6G as the donor and the acceptor, respectively, which takes advantage of the large Stokes shift of luciferin to avoid potential cross excitation of the acceptor. Their work leads to the photonic devices using biosynthesized materials as the gain medium and optofluidic intra-cavity bio/chemical sensing. © 2013 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4807837>]

An optofluidic laser integrates microfluidics with a laser cavity.^{1–4} It employs the gain medium in liquid for dynamic spectral tunability and broad spectral coverage. Meanwhile, due to its liquid handling and delivery capability, the optofluidic laser is uniquely positioned for biochemical and biomedical analysis. To date, most optofluidic lasers have been realized using organic dyes as the gain medium. Those dyes are either dissolved in free solution or conjugated with biomolecules such as DNA.^{5–12} Recently, the optofluidic laser based on fluorescent protein *in-vitro* and inside a live cell, as well as vitamin, has been demonstrated.^{13–16} The importance of using biosynthesized materials lies in two aspects. First, biosynthesized materials provide a new type of gain medium that may extend the utilities (such as spectral coverage) of existing organic dyes. More significantly, they can be synthesized through programmable and controlled biological processes and may be localized within certain regions (such as inside a cell) difficult to access with the conventional fluorophore labeling or conjugation method. Second, using biosynthesized materials in the optofluidic laser enables direct analysis of biomolecules and cells without external conjugation processes that may interfere with or even be detrimental to functionalities of the subject under study.

In this letter, we investigate the optofluidic laser using luciferin as part of the gain medium. Luciferins are a class of light-emitting compounds that can be synthesized by many different organisms such as fireflies, bacteria, and marine species.¹⁷ The bioluminescence mechanism of luciferins has widely been investigated in the past few decades^{18–24} and long been used in many bioassays, such as bioluminescence resonance energy transfer.²⁵ However, the applications of luciferins as laser gain medium have not been explored yet. In our current work, we use D-luciferin or firefly luciferin (see Fig. 1(a) for its molecular structure), which has a high luminescence quantum yield of $41.0 \pm 7.4\%$ ²³ and is one of the most commonly used substrates for bioluminescence reaction.

In our experiment, 1×10^{-2} M aqueous D-luciferin stock solution is prepared by dissolving D-luciferin sodium salt (L6882, Sigma) with MilliQ water. Then GTA buffer solution prepared by mixing 0.1 M 3,3-dimethylglutaric acid (Sigma), 0.1 M tris(hydroxymethyl)aminomethane (Sigma), and 0.1 M 2-amino-2-methyl-1,3-propanediol (Sigma) is added to achieve the desired D-luciferin concentration. The pH can be adjusted by adding HCl or NaOH to the GTA buffer.

We achieve the luciferin laser using an optofluidic ring resonator (OFRR) based on a thin-walled fused silica capillary with an outer diameter of $80 \mu\text{m}$ and wall thickness of $2 \mu\text{m}$. The circular cross section of the OFRR forms the ring resonator that supports the whispering gallery modes (WGMs) of high Q factors ($>10^6$). The WGM interacts evanescently with the gain medium and provides the optical feedback for lasing action. The experimental setup is illustrated in Fig. 1(b). The luciferin solution is flowed through the capillary at a flow rate of $1 \mu\text{l}/\text{min}$ and is pumped by an optical parametric oscillator (OPO) at 420 nm (5 ns pulse width and 20 Hz repetition rate). The emission is collected by a multimode fiber and subsequently transmitted to a spectrometer with a spectral resolution of 0.4 nm .

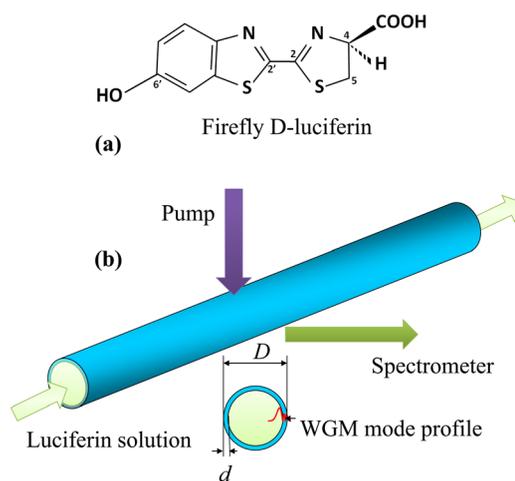


FIG. 1. (a) Molecular structure of firefly D-luciferin. (b) Schematic of the luciferin laser based on the OFRR. $D = 80 \mu\text{m}$, $d = 2 \mu\text{m}$.

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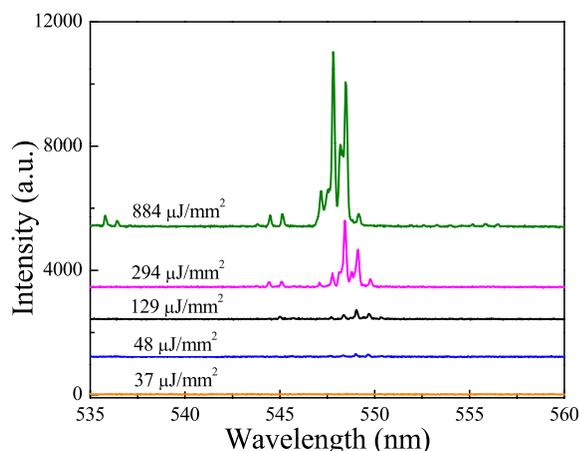


FIG. 2. Lasing spectra of 1 mM D-luciferin at pH 10.5 in the GTA buffer under various pumped energy densities. Curves are vertically shifted for clarity.

Since the quantum yield of luciferin in alkaline solutions is higher than that in acidic and neutral solutions,²⁶ we start by choosing the luciferin solution with pH 10.5 as the gain medium in the experiments. Fig. 2 shows the lasing spectra of 1 mM D-luciferin at pH 10.5 in the GTA buffer under various pump energy densities. Below the lasing threshold, only a broadband luciferin fluorescence emission centered around 545 nm can be observed. With the increased pump energy density, the luciferin laser starts to emerge at the longer wavelength side of the luciferin spectrum (~ 549 nm). The lasing threshold is estimated to be $39 \mu\text{J}/\text{mm}^2$ (see Fig. 3). When the pump energy continues to increase ($>129 \mu\text{J}/\text{mm}^2$), the lasing peaks blue-shift towards the fluorescence peak at 545 nm and new lasing peaks also appears around 535 nm. Note that the absorption spectrum of D-luciferin peaks at 390 nm in alkaline solutions, the lower absorption coefficient at the pump wavelength of 420 nm (the lowest attainable wavelength of the OPO) prevents the OFRR laser from achieving an even lower threshold.

To further investigate the pH dependence of the lasing threshold of the luciferin laser, in Fig. 3, we compare the

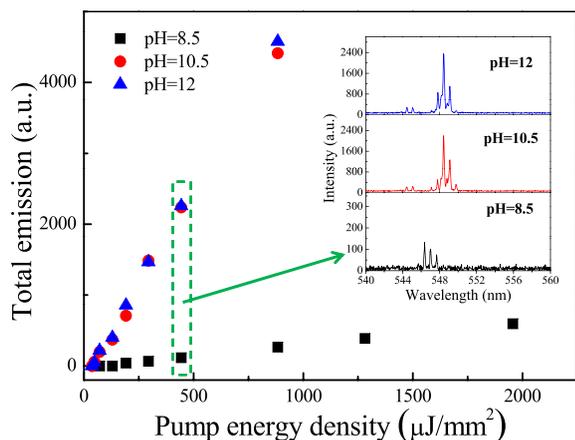


FIG. 3. Spectrally integrated laser output as a function of pump energy density for 1 mM D-luciferin at various pH values: square: pH 8.5; circle: pH 10.5; triangle: pH 12. The lasing threshold is 77, 39, and $38 \mu\text{J}/\text{mm}^2$ for at pH=8.5, 10.5, and 12, respectively. Spectral integration ranges from 535 nm to 550 nm. Inset, lasing spectra of the luciferin at various pH values under the same pump energy density of $294 \mu\text{J}/\text{mm}^2$.

lasing properties of 1 mM luciferin solutions with pH 8.5, 10.5, and 12, respectively. The pump wavelength is set at 420 nm. For the luciferin solution at pH 8.5, the lasing threshold is $77 \mu\text{J}/\text{mm}^2$, which is about twice that at pH 10.5 ($39 \mu\text{J}/\text{mm}^2$) and 12 ($38 \mu\text{J}/\text{mm}^2$), respectively. The higher threshold pH 8.5 is due to the lower fluorescence quantum yield and the lower absorption coefficient as the absorption peak of D-luciferin in pH 8.5 solutions moves to 330 nm, much shorter than 390 nm in alkaline solutions (pH > 10). In contrast, the threshold with pH 12 is even lower, indicating that the laser tends to be generated in alkaline conditions and the lasing efficiency is proportional to the pH values of the solution. Although the lasing threshold varies with the pH value of the solution, the observed lasing wavelengths for the three luciferin solutions remain in the same range from 545 nm to 550 nm, suggesting that lasing emission of D-luciferin is spectrally stable, in agreement with the previous studies of the fluorescence properties of D-luciferin.²⁶

We further achieve luciferin lasing at a lower concentration of 0.25 mM with pH 12. As shown in Fig. 4, the luciferin lasing peaks can be clearly observed when the pump energy density is $444 \mu\text{J}/\text{mm}^2$. The inset plots the spectrally integrated laser output as a function of pump energy density, showing a lasing threshold of $76 \mu\text{J}/\text{mm}^2$, even lower than that of 1 mM solution with pH 8.5.

Normalized absorption and fluorescence spectra of D-luciferin in the GTA buffer at pH 12 are shown in Fig. 5(a). The absorption spectrum has a peak at around 390 nm, which is in agreement with the previous studies of D-luciferin in alkaline solutions.²⁶ Its fluorescence spectrum with the excitation wavelength of 420 nm shows a peak at around 545 nm. Therefore, D-luciferin can be an excellent candidate for donor in energy transfer analysis due to its large Stokes shift (~ 155 nm), thus, avoiding cross excitation of the acceptor. In Fig. 5(b), we demonstrate an optofluidic fluorescence resonance energy transfer (FRET) laser by using D-luciferin and Rhodamine 6G (R6G) as the donor and acceptor, respectively. First, 5 mM pure D-luciferin solution at pH 12 is passed through an OFRR and pumped at 420 nm.

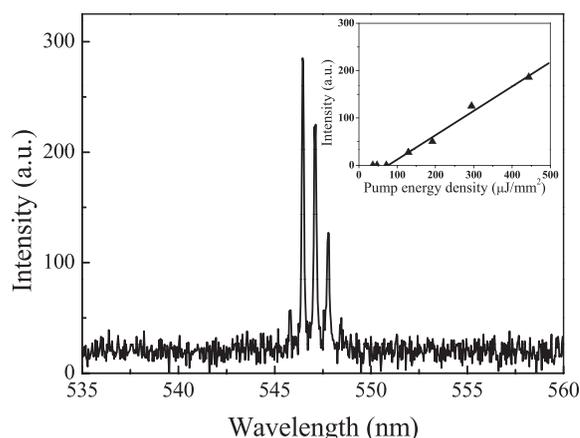


FIG. 4. Lasing spectrum of 0.25 mM D-luciferin at pH 12 under a pump energy density of $444 \mu\text{J}/\text{mm}^2$. Inset, spectrally integrated laser output as a function of pump energy density. Solid line is a linear fit above the lasing threshold of $76 \mu\text{J}/\text{mm}^2$. Spectral integration ranges from 535 nm to 550 nm.

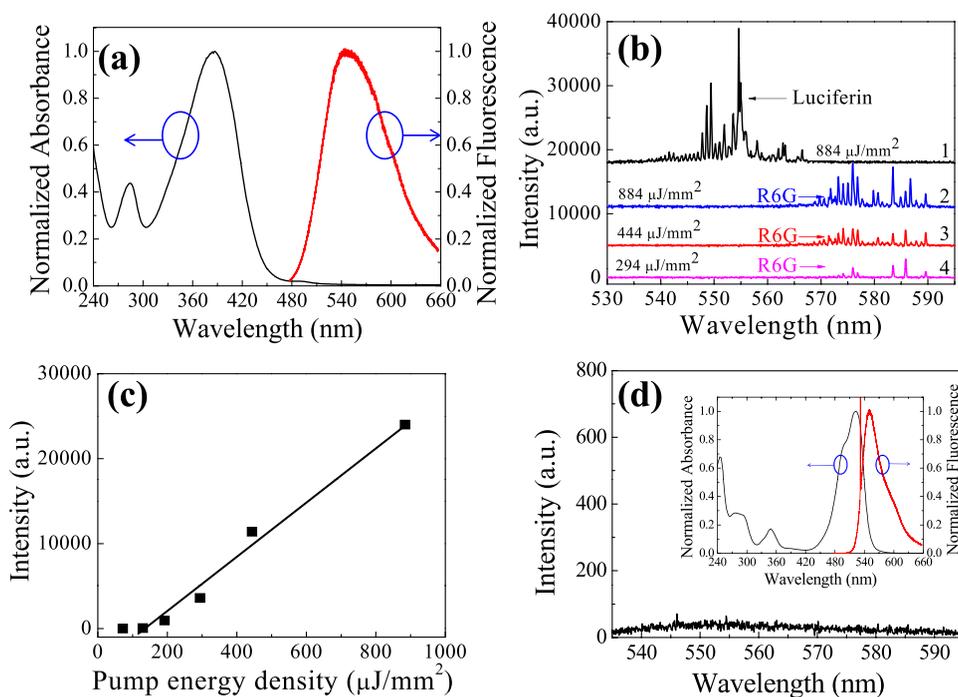


FIG. 5. (a) Normalized absorption and fluorescence spectra of D-luciferin in the GTA buffer at pH 12. Excitation wavelength for the fluorescence spectrum is 420 nm. (b) Curve 1: Lasing spectrum of 5 mM D-luciferin in the absence of R6G. Curves 2–4: FRET lasing spectra of R6G under various pump energy densities. The concentration of the donor (D-luciferin) and the acceptor (R6G) are 5 mM and 0.1 mM, respectively. Excitation wavelength is 420 nm. Curves are vertically shifted for clarity. (c) FRET laser output from R6G as a function of pump energy density. Solid line is a linear fit above the lasing threshold of 124 $\mu\text{J}/\text{mm}^2$. (d) Fluorescence spectrum of 0.1 mM R6G in the GTA buffer at pH 12 when the pump energy density is 2 mJ/mm^2 and excitation wavelength is 420 nm. Inset: Normalized absorption and fluorescence spectra of R6G in the same buffer. Excitation wavelength for the fluorescence spectrum is 532 nm.

Typical lasing spectrum for D-luciferin is observed, as shown in curve 1 of Fig. 5(b). Then, the mixture of luciferin (5 mM) and R6G (0.1 mM) is flowed through the capillary and pumped at the same wavelength of 420 nm. In the presence of R6G, the lasing peaks around 590 nm emerge, which are within the expected lasing emission of the acceptor.⁷ With the increased pump energy density, the lasing spectra ranging from 570 nm to 590 nm can be observed (curves 2–4 in Fig. 5(b)). Fig. 5(c) plots FRET laser output from R6G as a function of pump energy density, showing a FRET lasing threshold of 124 $\mu\text{J}/\text{mm}^2$. Note that the lasing from luciferin is not observed even at a high pump level (884 $\mu\text{J}/\text{mm}^2$), which indicates the donor lasing is completely quenched and the energy is transferred to the acceptor with high efficiency. As a control experiment, 0.1 mM pure R6G solution is used and pumped at 420 nm. Due to the low absorption coefficient of R6G at 420 nm, only weak fluorescence spectrum as shown in Fig. 5(d) can be observed even at an extremely high pump energy density of 2 mJ/mm^2 , thus, eliminating cross laser excitation of the acceptor.

In summary, we have demonstrated an OFRR optofluidic laser with luciferin as the gain medium. The laser characteristics under different lasing conditions (solution pH value, gain medium concentration, and presence of the acceptor) are investigated. This work will lead to the development of biomaterials for laser gain medium and bio-inspired photonic devices. In particular, as compared to fluorescent proteins,²⁷ the large Stokes shift of luciferin, which avoids undesirable acceptor cross excitation, makes it attractive in optofluidic FRET lasers for ultrasensitive laser intra-cavity bio/chemical sensing.^{12,15}

The work was supported by the National Science Foundation under ECCS-1045621 and CBET-1037097, and by the National Natural Science Foundation of China under

61128011. X.W. was supported by China Scholarship Council (No. 201208310235) and by the University of Michigan.

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