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Detection system for *Legionella* bacteria using photogate-type optical sensor

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This study describes a bacterial sensing system using a photogate-type fluorescence sensor for the purpose of identifying *Legionella* bacteria, which are known to emit different fluorescence for each bacterial species when irradiated with UV excitation light. First a weak-photocurrent measurement system in combination with a photogate-type optical sensor and a circuit board which was mounted with a current–voltage converter, an analog-to-digital converter, and a microcomputer was fabricated. Then weak-light measurement was performed by simulating the bacterial fluorescence of *Legionella*. A difference in the current ratio generated in the sensor was found depending on the wavelength of the LED light source, showing that the system works as a spectrometer. Finally, the system was applied to measure the fluorescence of the species of *Legionella dumoffii* and *Legionella erythra* and each species of *Legionella* was identified just by obtaining the current ratio.

1. Introduction

It is well known that an outbreak of *Legionella* infections can cause Legionnaires' disease such as Pontiac fever and Legionnaires' pneumonia.[1–6)] To avoid such an outbreak of bacterial infection, one possible solution is finding harmful bacteria before infectious disease spreads. Bacteria sensing such as in the medical front is thus attracting a great deal of interest. A sensor is now expected to be developed that can detect bacteria in a shorter time and give a warning when the amount of bacteria exceeds a certain standard.[7)] It is necessary to know in advance the parameters such as the fluorescence wavelength and the fluorescence intensity dependence on the excitation light irradiation time.[8–22)] In the course of our study, we have succeeded in observing the time dependence of the intensity of blue fluorescence from *Legionella dumoffii (L. dumoffii)*[23,24)] and red fluorescence from *Legionella erythra (L. erythra)*[25)] using a photogate-type fluorescence sensor.[20)] Although an optical filter for bacterial fluorescence discrimination is required to observe the characteristic fluorescence of *L. erythra*, which emits long-wavelength red fluorescence, we have demonstrated the possibility of discriminating the species by simultaneously acquiring the surface-side and substrate-side currents of the sensor.[22)] This study describes the measurement method, which does not use an optical filter for bacterial fluorescence discrimination but uses just a photogate-type fluorescence sensor to identify *Legionella* species known to emit different fluorescence when irradiated with UV excitation light. In addition, we show a miniaturized bacterial fluorescence measurement system that is portable and can replace the large devices such as fluorescence microscopes, spectrometers, and semiconductor parameter analyzers that have been used in the past.

2. Experimental

2.1. Photogate-type optical sensor

We have proposed a method of fluorescence detection for *Legionella* bacteria by combining a MEMS channel that traps *Legionella* with a photogate-type optical sensor.[16,17,20–22)] Figure 1(a) shows a schematic cross-sectional view of the photogate-type optical sensor. *Legionella* bacteria emit fluorescence when irradiated with this UV light with a wavelength of 365 nm. The fluorescence entering the sensing area of the sensor generates photoelectrons, which are read as photocurrent to detect the fluorescence.[26,27)] The fluorescence of *Legionella* irradiated by UV light enters the sensing area and generates photoelectrons. The fluorescence is detected by reading the photoelectrons as an electric current as expressed by Eq. (1). Figure 1(b) schematically shows the potential in the depth direction from the photogate and Si interface in the sensing area. Conventionally, the current flowing at the interface side of the photogate, $I_{\text{p-well}}$ [Fig. 1(a)], is measured around the peak of the potential formed by applying the gate voltage V_{PG}.

$$I = \frac{qS\lambda}{hc}(1 - e^{-\alpha W})\phi_0 \tag{1}$$

q: charge element, S: photosensitive area, λ: wavelength,
h: Planck constant, c: speed of light in vacuum,
α: absorption coefficient of light of wavelength λ into Si,
W: penetration depth of light of wavelength λ into Si,
ϕ_0: light intensity at the surface of the receiver

As shown in Fig. 1(c), the penetration depth of light into Si varies with wavelength, with shorter wavelengths penetrating shallower and longer wavelengths penetrating deeper.[28)] The depth of each well of the sensor was designed to be 7 μm for the n-well and 4 μm for the p-well, and the sensing area size was 300 μm pixels.[29)] By measuring the photocurrent $I_{\text{n-well}}$ [Fig. 1(a)] at the substrate side as a measurement which takes advantage of the different light penetration depth characteristics for each wavelength, we can obtain information reflecting the behavior of fluorescence at longer wavelengths penetrating deeply. In this study, as a new detection method for *Legionella*, we observe the photocurrent, both $I_{\text{p-well}}$ and $I_{\text{n-well}}$, of a photogate-type optical sensor without optical filters for bacterial fluorescence discrimination to clarify the characteristic fluorescence properties of this bacterium. Hereafter, for simplicity, $I_{\text{p-well}}$ and $I_{\text{n-well}}$ are respectively referred to as I_{out} and I_{sub}.

2.2. Small measurement system

In this section, we describe the configuration and fabrication of a small measurement system with excellent portability.

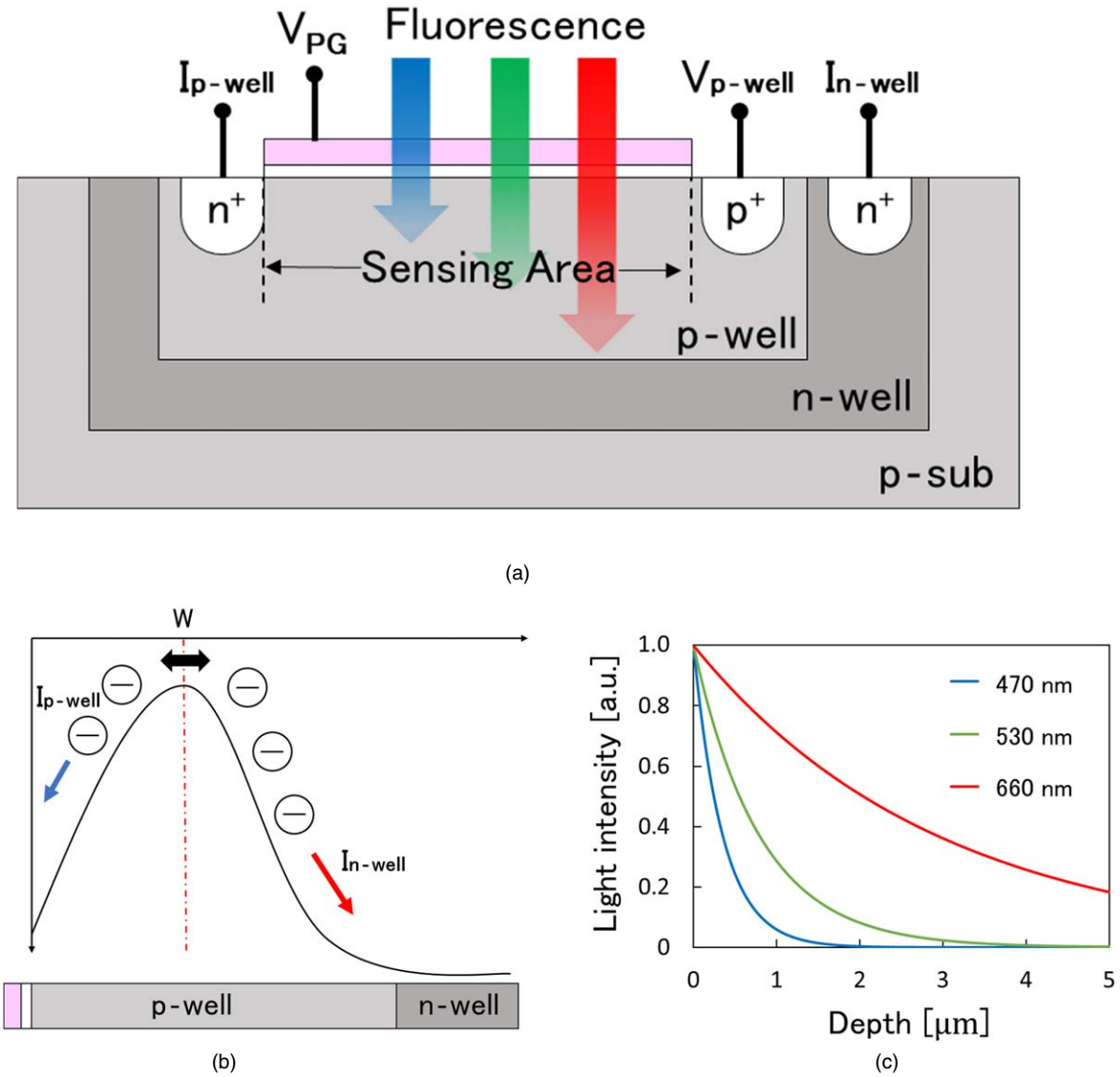

Fig. 1. (Color online) Photogate-type optical sensor: (a) cross-sectional diagram, (b) schematic diagram of potential, (c) light intensity attenuation characteristic versus light penetration depth.

The entire system is shown in Fig. 2. The sensor output can be easily checked with a PC monitor through an *I–V* conversion circuit using an operational amplifier, an AD converter, and a microcontroller. The resolution of the current value of the sensor output response was set to a 20 nA dynamic range and 1.25 pA resolution to measure the weak fluorescence of *Legionella*. The fabricated system is shown in Fig. 3. The dimensions are about 3.5 cm × 13 cm. Each module was mounted on a printed circuit board. The dynamic range at a photogate voltage of 3 V was measured when an LED light source with two wavelengths of 470 nm and 660 nm was irradiated through a 400 μm diameter optical fiber and a 20× objective lens. As shown in Fig. 4, the output voltage is proportional to the light power for both blue (470 nm) and red (660 nm) light. Next, assuming the red fluorescence of *L. erythra*, we simultaneously irradiated UV excitation light of 365 nm and weak light of 660 nm to simulate the actual measurement conditions. Figure 5 shows the measurement results of the simulated experiment. These results confirm the linearity of the sensor output to light

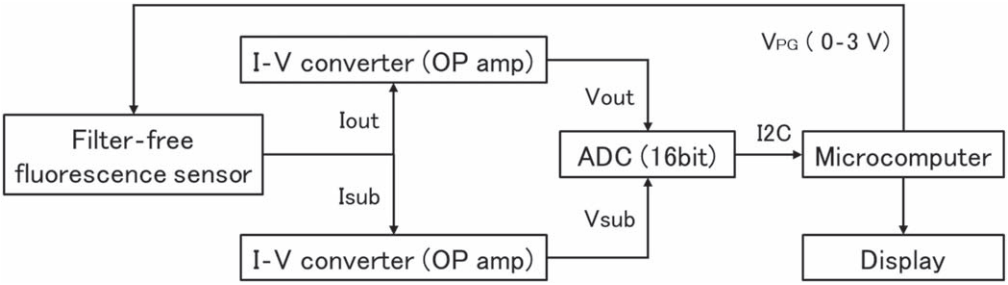

Fig. 2. Configuration of a compact measurement system.

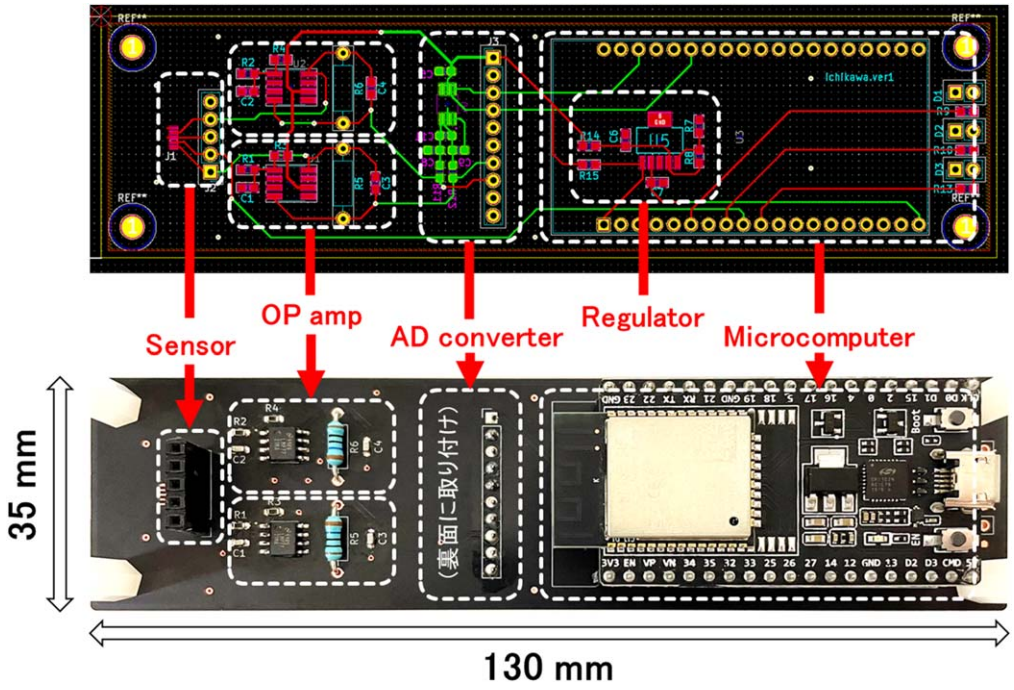

Fig. 3. (Color online) Photo of fabricated circuits.

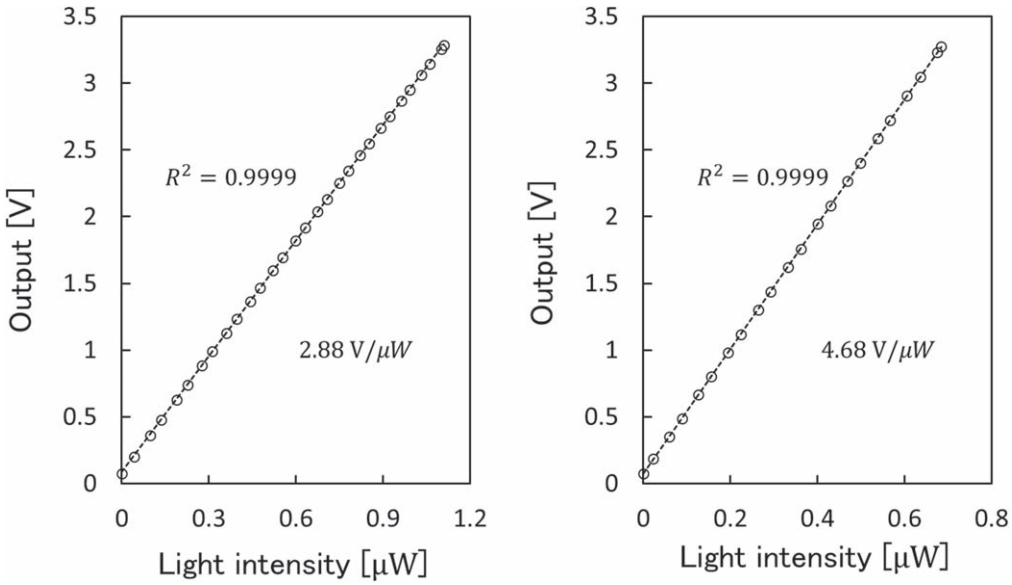

Fig. 4. Output voltage versus input power for blue (left) and red (right) light. The applied photogate voltage was 3 V. An LED light source with wavelengths of 470 nm and 660 nm was irradiated through a 400 μm diameter optical fiber and a 20× objective lens.

intensity and show that it is possible to measure changes in light as weak as the fluorescence emitted from bacteria.

2.3. Experimental system

Figure 6 shows a schematic diagram of the experimental system used in this study. Cultured *L. erythra* was placed in a PDMS channel with a cavity of 1 cm × 1 cm and 30 μm depth, covered with glass, and placed on top of the photogate-type optical sensor. This photogated optical sensor was fabricated at the Toyohashi University of Technology.[29] UV excitation light was irradiated through a fluorescence microscope (BX43, Olympus). In this experiment, fluorescence spectra were obtained using the fluorescence microscope and a spectrometer (USB4000, Ocean Optics). At the same time, photocurrent was measured with the combination of the photogate-type optical sensor and the circuit. To prevent the excitation UV light scattered by the *Legionella* cells from exposing unexpected places on the sensor chip, such as interconnection pads, an optical filter that cuts off excitation UV light was placed between the glass slide and the sensor. Figure 7 shows the designed and fabricated *Legionella* detection system. The system consists of a frame, a PDMS microfluidic, and a photogate-type optical sensor.

3. Results and discussion

3.1. Spectral observation of fluorescence from *Legionella*

Figure 8 shows the fluorescence spectra of *L. dumoffii* obtained with the spectroscope from just after irradiation with 365 nm UV excitation light to 180 s. In the case of *L. dumoffii*, fluorescence with a peak around 490 nm is observed immediately after UV excitation light irradiation and decays

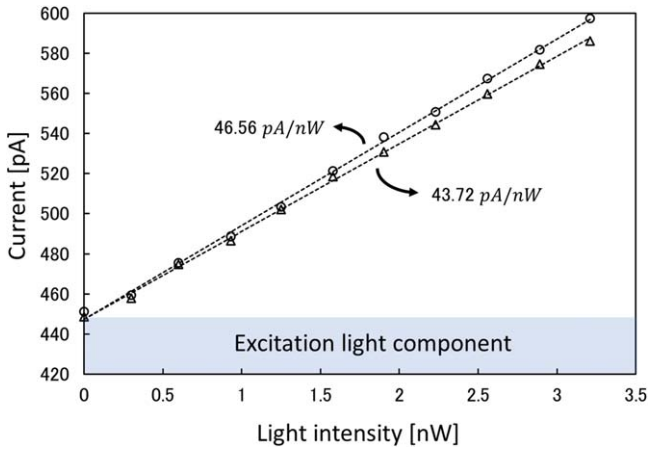

Fig. 5. (Color online) Incident light intensity versus output characteristics when simulating fluorescence with an LED light source ($\lambda_{365\ nm}$, 1 nW + $\lambda_{660\ nm}$, 0–3 nW). The open circles and open triangles stand for the cases with photogate voltages of 3 V and 1 V, respectively.

monotonically.[20,21] This is photobleaching and corresponds to the observation of the fading process of fluorescent substances in the cells of *L. erythra* due to photochemical reactions caused by UV light.[30] In the fluorescence spectra of *L. erythra* shown in Fig. 9, two phenomena, decay and increase of red fluorescence, are observed: the decay is also photobleaching and the increase of a peak around 676 nm, which is not observed immediately after UV excitation light irradiation, is considered to be a kind of expression of bacterial stress response,[19] which we have also reported for *Legionella pneumophila*, and is thought to be an observation of the production process of new fluorescent substances.[8–15] In the following, we describe the possibility of discriminating between *Legionella* species by using the ratio of the photocurrent I_{sub} to I_{out} of the photogate without spectral observation.

3.2. Measurement of photocurrent with the developed system

Figure 10 shows the ratios between I_{out} and I_{sub} when the sensor was exposed to four different light source wavelengths of 470 nm, 530 nm, 590 nm, and 680 nm. In this study, a gate voltage of 3 V was applied to the photogate and the potential peak position was formed at about 1 μm from the Si surface.[29] This result confirms that different ratios can be obtained depending on the wavelength of the light, showing that the simultaneous measuring of currents, I_{out} and I_{sub}, of the photogate works as a kind of spectrometer. We measured the photocurrents of I_{out} and I_{sub} simultaneously for both *L. dumoffii* and *L. erythra*. The sample preparation protocol for each experiment was as follows: Cultivated *Legionella* about 5 mg was collected from culture media by a platinum loop and then spread over a glass slide before the fluorescence observation. The population of *Legionella* cells was estimated to be about 60 cells/100 μm^2 from the number of *Legionella* cells counted, which emitted fluorescence in the field of the fluorescence microscope. The current ratios of *L. dumoffii* and *L. erythra* were, respectively, around 0.78 and 2.80, as shown in Fig. 11, indicating that fluorescence with shorter wavelengths from *L. dumoffii* and fluorescence with longer wavelengths from *L. erythra* were observed. The obtained ratios were reproducible in five measurements under the same measurement conditions. These results suggest that it is possible to discriminate between *Legionella* species with different fluorescent wavelength bands from the current ratio by measuring the fluorescence emission from *Legionella* under UV excitation light using this system. We confirmed the possibility of obtaining wavelength information and identifying *Legionella* species with different fluorescence wavelengths by observing the ratio of I_{out} to I_{sub} described above; the ratio obtained from *Legionella* is however still a little bit different from that shown in Fig. 10. Furthermore, we were not able to observe a specific peak growth corresponding to the peak at 676 nm for *L. erythra*

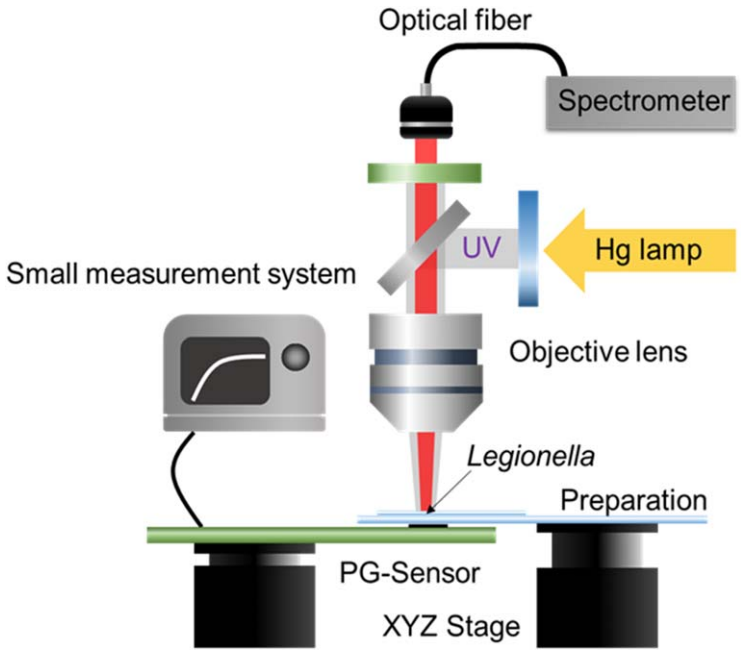

Fig. 6. (Color online) Experimental setup.

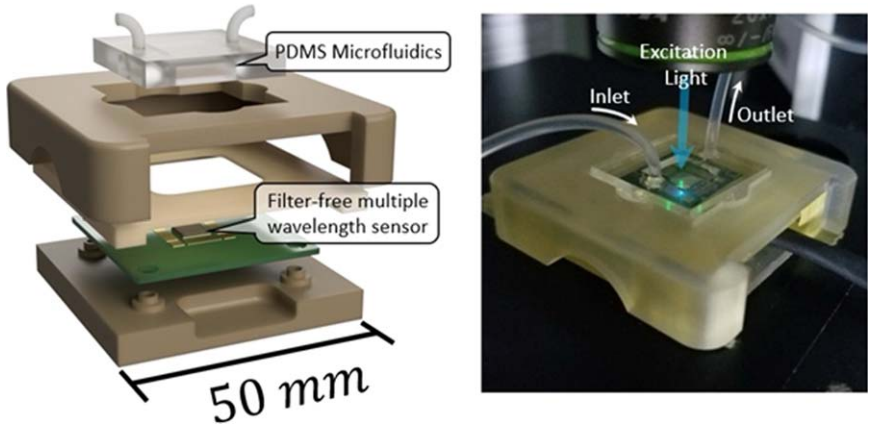

Fig. 7. (Color online) Photos of the 3D design (left) and the fabricated bacteria detection system (right).

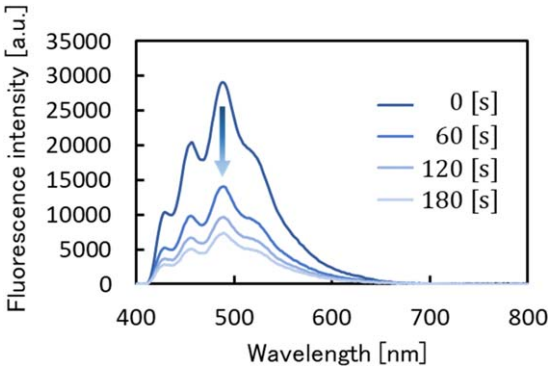

Fig. 8. (Color online) Fluorescence spectra of *L. dumoffii* with the parameter of UV light irradiation time.

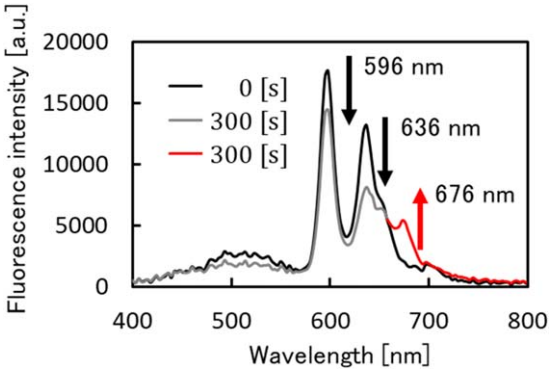

Fig. 9. (Color online) Fluorescence spectra of *L. erythra* with the parameter of UV light irradiation time.

as shown in Fig. 9. Although further study is necessary to overcome the issues mentioned above, the small fluorescent observation system we have developed paves the way for sensing pathogenic bacteria.

4. Conclusions

We investigated a method using a photogate-type optical sensor as a detection and identification system for *Legionella* bacteria. The present results validate a compact measurement system for *Legionella* and show its applicability to bacterial measurement systems by demonstrating a difference in resolution for light intensity changes on the order of nanowatts and a current ratio dependence on wavelength, which is comparable to the fluorescence of *Legionella*.

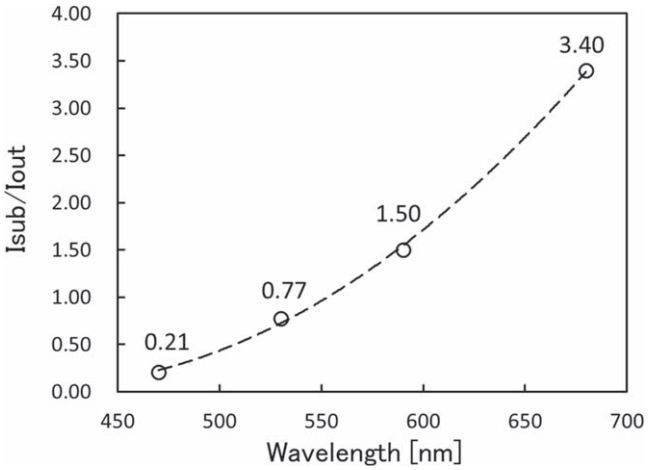

Fig. 10. Current ratio versus wavelength.

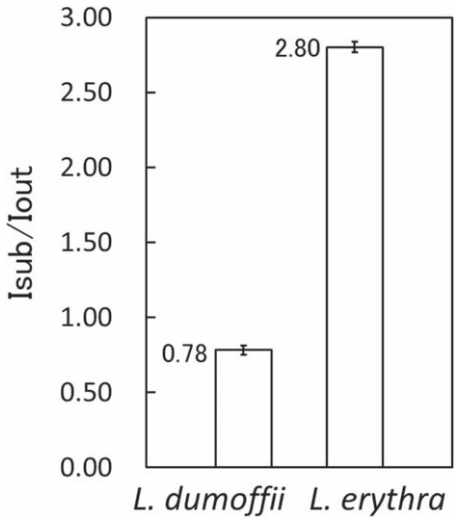

Fig. 11. Measured current ratio for two types of *Legionella*.

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