集積化MEMS技術による細菌のストレス応答解明と細菌同定技術への応用

Identification of *Legionella* Species by Photogate-Type Optical Sensor

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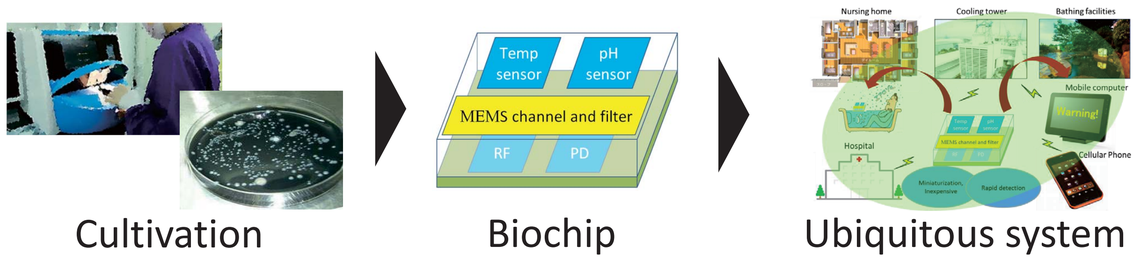
Summary

This work describes simple method for sensing bacteria, *Legionella* by using a photogate-type optical sensor and MEMS chip. We observed the behavior of fluorescence from *Legionella dumoffii* (*L. dumoffii*) and *Legionella erythra* (*L. erythra*) when they are exposed to ultraviolet light. The time dependence of the fluorescence intensity of *L. dumoffii* and *L. erythra* obtained with photogate-type optical sensor agreed well with that of fluorescence intensity obtained with spectrometer, showing that each *Legionella* is able to be detected by photogate-type optical sensor. Using the difference of their behavior, the identification of each bacterium was successfully performed for a mixed bacterial system of *L. dumoffii* and *L. erythra* by the combination of photogate-type optical sensor and optical filters.

Introduction

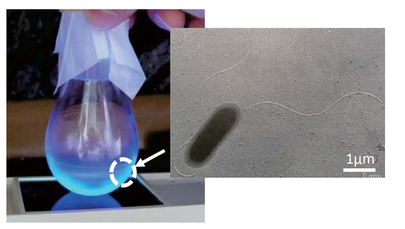
A Sensor for avoiding an outbreak of bacterial infection



Cultivation　Biochip　Ubiquitous system

Development of a sensor that can detects bacteria without cultivation in a short time is desired.

What is *Legionella* ?



[1] Ed. by S.Yoshida, Y.Yanagi, Y.Yoshikai:「Toda's New Bacteriology」Nanzando, 2013

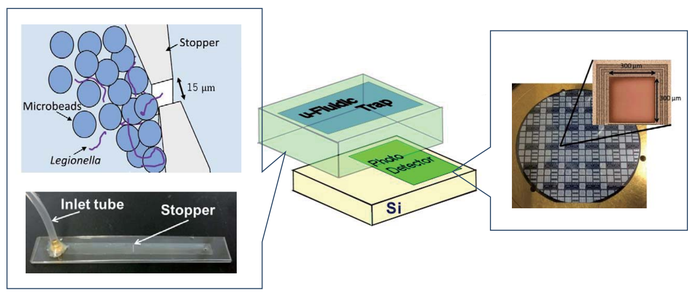
Size : length 1-10μm, diameter 0.3-0.9μm
Infection : pneumonia
Habitat : Facilities using circulating water circulating water
Characteristic : Fluorescence emission by ultraviolet light irradiation
Definitive diagnosis : Culture method

Method

The combination of photogate-type optical sensor and MEMS Chip

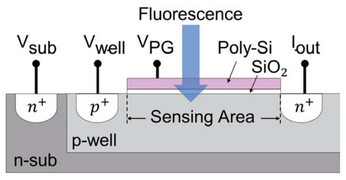
Procedure

1. Put bacteria with beads in the chip
2. Set the chip on the sensor
3. UV irradiation
4. *Legionella* emits fluorescence
5. Detect fluorescence by the sensor

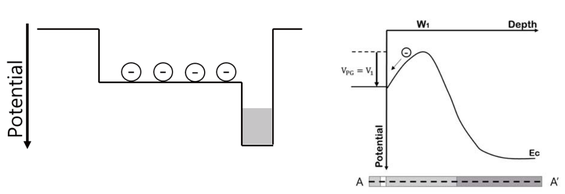


Photogate-type optical sensor

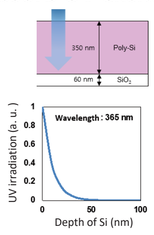
- **Schematic cross-section**



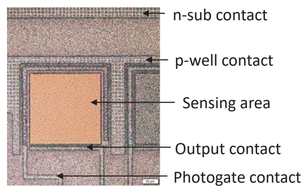
- **Potential diagram of the sensor**



- **Absorb UV excitation**



- **Bird's eye view**



- **Photocurrent produced by Legionella fluorescence**

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

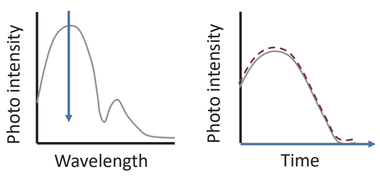
ϕ : Intensity of light　*a : Absorption coefficient*
W : Depth　*s : Sensing area*
λ : Wavelength　*h : Planck's constant*
I : Photocurrent　*q : Elementary charge*

It is necessary to know characteristic parameters: wavelength λ and intensity $\phi_0(t)$.

How to identify ?

- **Use λ and rate constants**

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



$$B(t) = A_0 \times \frac{k_1}{k_2 - k_1}(exp(-k_1 t) - exp(-k_2 t))$$

Bacteria 1(λ..., $\phi_0(t) \cong k_1 ..., k_2 ...$)
Bacteria 2(λ..., $\phi_0(t) \cong k_1 ..., k_2 ...$) ← Compare

- **Chemical kinetic analysis**

Starting material　Fluorescent material　The reaction product
Changes in substances inside bacteria　A $\xrightarrow{k_1}$ B $\xrightarrow{k_2}$ C

Rate equation

$$\frac{dA}{dt} = -k_1 \times A$$

$$\frac{dB}{dt} = k_1 \times A - k_2 \times B$$

$$\frac{dC}{dt} = k_2 \times B$$

Boundary condition :
Break nutrients in a pure water
When t→∞, A=B=0, C=A_0

Time change of each substance

$$A(t) = A_0 \times exp(-k_1 t)$$

$$B(t) = A_0 \times \frac{k_1}{k_2 - k_1}(exp(-k_1 t) - exp(-k_2 t))$$

$$C(t) = A_0 \times \frac{k_1 k_2}{k_2 - k_1}\left(\frac{1}{k_2} \times exp(-k_2 t) - \frac{1}{k_1} \times exp(-k_1 t)\right) + A_0$$

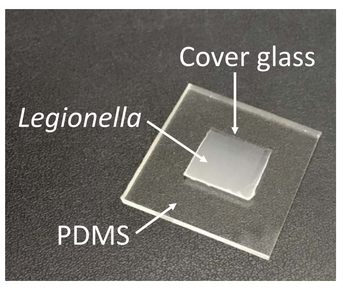
Experiment Results

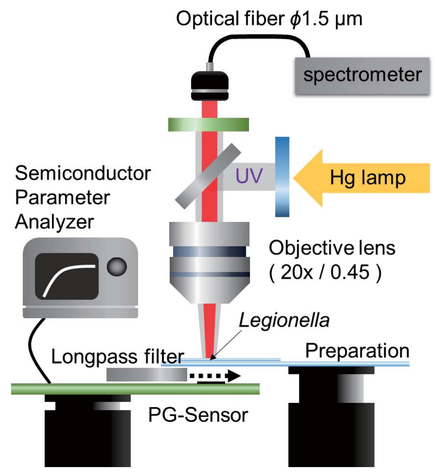
Identification of *Legionell*a species

This work describes the following two investigations:
(1) Observation of respective fluorescence from *L. dumoffii* and *L. erythra* by the sensor when they are exposed to UV light.
(2) Discrimination of *L. erythra* from a mixed system of *L. dumoffii* and *L. erythra* by using the combination of PG-sensor and optical filters.

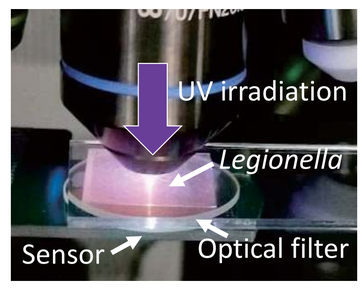
Measurement system

- Applied voltage -
Vsub, VPG, Iout・・・3V
Vwell ・・・GND



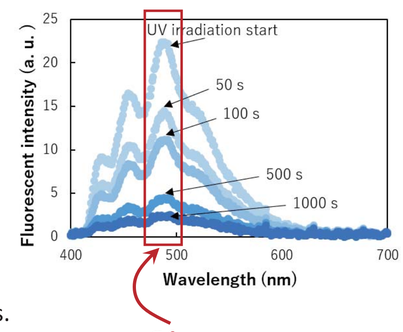


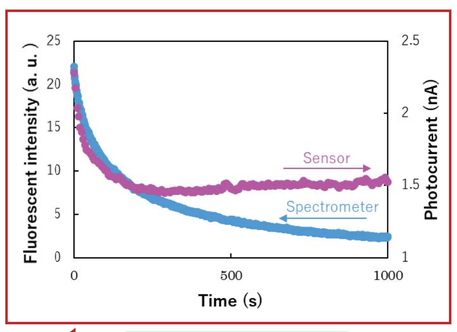
- Irradiation -
1.2 mW/cm2
365 nm



L. dumoffii : blue fluorescence

λ...490 nm, $\phi_0(t) \cong k_1 ..., k_2 ...$



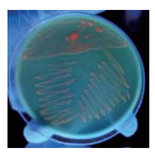


- The spectra have 3 clear peaks.
- We observed the photobleaching process of Legioliulin.

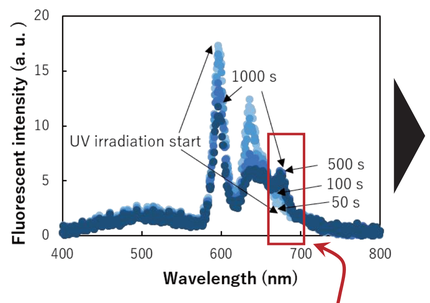
$$I = \frac{qS\lambda}{hc}(1 - e^{-\alpha W})\phi_0(t)$$

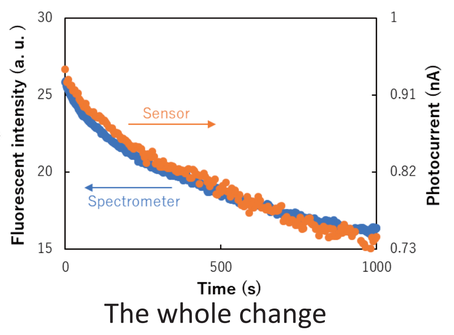
Rate constants
Spectrometer k_2...0.023
Sensor k_2...0.021

L. erythra : red fluorescence



λ...677 nm, $\phi_0(t) \cong k_1 ..., k_2 ...$



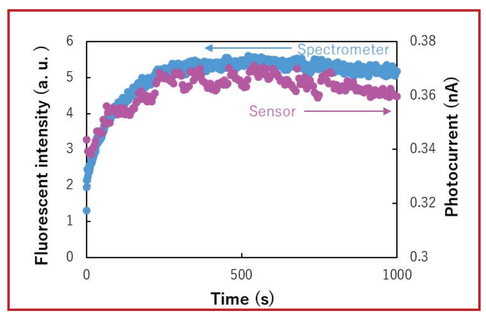


The whole change

- The spectra have 4 clear peaks
- We observed the photobleaching process at 500, 600, 640 nm
- We observed the increases at 677 nm. This increase is probably due to the production of new fluorescent material.

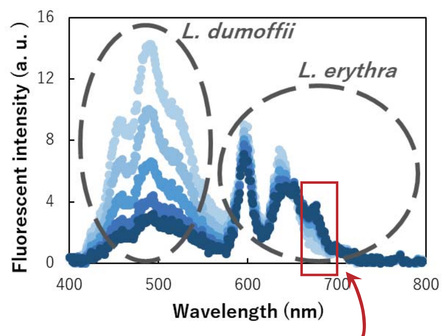
$$I = \frac{qS\lambda}{hc}(1 - e^{-\alpha W})\phi_0(t)$$

Rate constants
Spectrometer k_1...0.0013, k_2...0.0028
Sensor k_1...0.0019, k_2...0.0022

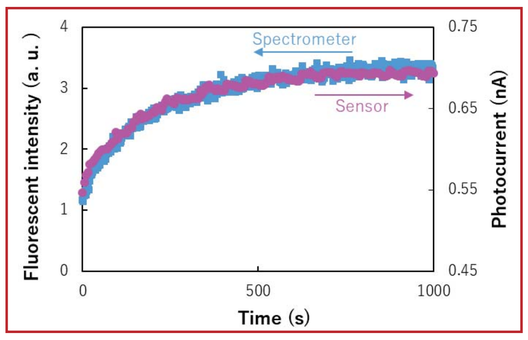


With a long pass optical filter

Mixture of *L. dumoffii* & *L. erythra*



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



Rate constants
Spectrometer k_1...0.0012, k_2...0.0014
Sensor k_1...0.0013, k_2...0.0014

L. erythra cells in the bacterial mixed system were identified by using a combination of the sensor and an optical filter.