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A portable fluorescent sensor for on-site detection of microalgae

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ABSTRACT

This work reports the development of a portable and low cost fluorescent sensing system with a disposable microfluidic chip for on-site detection of a microalgal sample and its concentration. The sensor system has multiple light emitting diodes (LEDs) for excitation and a photodetector for measuring a fluorescent signal from a microalgal sample. The concentration of a microalgal sample is determined by measuring the fluorescent signal emitted by chlorophyll *a*. A dichroic filter and a color filter are also added to allow only the fluorescent signal from chlorophyll *a* to pass through the photodetector. The microfluidic chip consists of a glass slide and a PDMS channel with a vacuum pump, which collects a small volume of the microalgal sample (<10 μl). The fluorescent sensor was characterized with varying concentration of microalgal samples and demonstrated its capability of measuring microalgal concentrations. The sensor was also tested with microalgal samples mixed with different turbidity water to validate its selectivity. The results show that the fluorescent detection of microalgal concentration is not influenced by the turbidity level of the sample solution. The developed system can be used for on-site screening and monitoring of microalgal population with an integrated excitation and detection circuitry.

1. Introduction

The lipids from microalgae have great potential to be used as a next generation renewable fuel [1–3]. In order to obtain the maximum microalgal production yield from the culturing system, environmental conditions of cultivating system must be optimized and controlled [4,5]. Therefore, an effective monitoring system is necessary to manage the microalgae cultivation system.

There are several techniques available to detect and to quantify microalgae in water. Optical and fluorescent microscopy techniques are manual cell counting methods that require a laboratory setup and laborious efforts [6]. Flow cytometry is a commonly used method to count microalgal cells providing an accurate result with rapid-analysis, however it is expensive and requires a skilled operator to acquire desirable performance [7]. An optical density technique is simple and yet provides an accurate result for the total suspended solids measurement in water [8,9]. However, it cannot differentiate between microalgal cells and debris in water possibly drawing an erroneous result.

Chlorophyll fluorescence offers many advantages over other detection methods in terms of accuracy, measurement time, and portability [10]. In addition, it is capable of differentiating microalgal cells from debris since fluorescence only comes from chlorophyll pigments. Several portable microalgal fluorescent sensors have been developed for the *in situ* monitoring purposes [11–17]. There are largely two types of portable chlorophyll fluorescence sensors—a submersible and a non-submersible type. A submersible sensor can detect and monitor microalgae at underwater environment, but it is not suitable for measuring a small amount of sample. A non-submersible fluorescent sensor is capable of measuring a small amount of sample but it is difficult to integrate into a real-time monitoring system.

Microfluidic technology has enabled the small sample volume requirement, shorter analysis time with a lower cost, and miniaturization. In order to develop a portable sensor device with a microfluidic PDMS chip, simple pumping method without any power source or tool is essential for the system. The passive pumping method of finger powered microfluidic pumping system, and the passive pumping lid system showed a good demonstration of

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power free portable microfluidic device [18,19]. However, these pumping methods require relatively difficult PDMS chip fabrication process, and low design freedom due to an additional apparatus such as a lid. The vacuum pumping method reported by Hosokawa et al. [20] and Liang et al. [21] is a convenient way to deliver a liquid sample without external or on-chip pumping devices. Xu et al. [22] suggested a syringe-assisted point-of-care pumping system to achieve better controllability compared to the vacuum pumping system and to remove the vacuum chamber treatment process. However, our sensor device requires minimum controllability during the sample loading process. Also, for the field application, the testing chip can be packaged in a vacuum sealer that is commonly used for medical devices. Before loading the sample, the sealer will be opened for vacuum pumping [21,23].

Here, we report a microalgal fluorescent sensor that is portable and non-submersible for quantifying the population of microalgal cells in an aliquot sample volume. The main purpose of our sensor is to use a field deployable sensor as well as an in-line sensor integratable to a massive microalgal cultivation system called the Hydraulically Integrated Serial Turbidostat Algal Reactor (HISTAR) system [24,25]. The sensor has an excitation and a detection apparatus in the same plane, which can easily be configured for either purpose. The turbidostat in the HISTAR system is necessary for monitoring the microalgae concentration and the contaminants in the culturing system. However the turbidostat does not differentiate contaminants from microalgae [26]. Thus, our microalgal fluorescent sensor will provide more accurate result of microalgal cell concentration of HISTAR system compared to the turbidostat. The turbidostat can be used only to measure the contaminants in water, since the fluorescent sensor cannot detect the non-fluorescence contaminants.

The working principle of chlorophyll fluorescence is detecting the emitted fluorescent light from chlorophyll molecules when they have absorbed the light. The *Chlorella vulgaris* that is the target species of interest has chlorophyll *a* as its major photosynthetic pigment [27]. To demonstrate the on-site microalgae fluorescent detection, we have utilized the fluorescent property of chlorophyll *a* pigment which has absorption wavelength of 440 nm-peak and emission wavelength of 680 nm-peak [17,28]. We have chosen the 448 nm wavelength LED light as an excitation light and a long pass color filter having cut-off at 645 nm. A dichroic filter has been added to reduce the noise. Since the light source and the detector with filters are mounted below the PDMS microfluidic chamber filled with a sample solution, the sensor can be easily integrated into an in-line monitoring setup (e.g., the HISTAR system) by simply removing the microfluidic chip and top cover and deploy it under the clear pipeline in the system. The microalgal population count value is compared with an optical density sensor measurement value to compare the accuracy and selectivity when debris and dirt are added into the microalgal sample solution.

2. Experimental methods

2.1. Detection principle

Fig. 1 shows the detection concept of the fluorescence sensor. The system consists of three different parts: light emission, detection, and sample handling. Philips Lumileds Rebel color LEDs were used for the light emission part. Philips Lumileds Rebel color LED (Philips, Netherlands) with 448 nm peak wavelength was selected to detect the microalgae which emits 680 nm fluorescent light from chlorophyll *a*. A photodiode (FDS100, Thorlabs, USA) with optical filters was placed below the PDMS microfluidic chamber to detect the fluorescent light signal from the sample. The first optical filter on the top side of the printed circuit board (PCB)

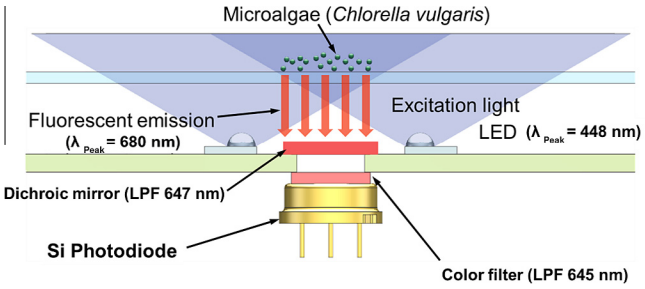

Fig. 1. Schematic illustration of the fluorescent microalgae detection system.

where all LEDs are mounted is a dichroic filter (PIXELTEQ, USA) working as low pass for 647 nm wavelength light. The second optical filter below the PCB is a color filter (Edmund Optics, USA) working as low pass for 650 nm wavelength fluorescent light. Microalgal sample solution is introduced into a disposable PDMS microfluidic chip placed on top of the LEDs.

2.2. System design configuration

The schematic configuration of the sensor system is illustrated in Fig. 2(a). The sensor jig was fabricated with a three dimensional (3-D) printer. Polylactic acid (PLA), a biodegradable thermoplastic material derived from renewable resources, was used as the structural material for 3-D printing. The photodetector and the color filter are mounted in the bottom cover ($90 \times 50 \times 20\ mm^3$) and covered with the PCB. The PCB's aperture was aligned with photodetector's window to receive the fluorescent signal from microalgal sample. Tray guide and the tray with a PDMS microfluidic chip containing the sample were aligned precisely to have maximum overlapping excitation light projected on the microfluidic chamber and to obtain maximum fluorescent light signal. The top cover is to achieve the fluorescence measurements in the dark environment. The top and the bottom cover block ambient light when fully assembled as shown in Fig. 2(a). The actual device is shown in Fig. 2(b).

2.3. PCB design

The PCB comprises six excitation LEDs with 448 (±10) nm peak wavelength. The aperture in the middle of the PCB allows for the fluorescent light from the microalgae to pass through to the photodetector. A dichroic mirror filter was installed on the top side of the PCB and a color filter was installed on the bottom side of the PCB to reduce the noise. Thelen and Chu [29] have demonstrated a portable low current sensing circuit design for a fluorescence optical detection. For a fully integrated system, a nanoampere range current meter for the photocurrent detection can be easily implemented and integrated with our proposed device for the portable detection of the microalgae.

2.4. Microfluidic chip

The microfluidic PDMS chip design is shown in Fig. 3(a). The dimension of the sensing chamber is 5 mm in diameter and 200 μm in thickness. The PDMS chip consists of a vacuum pumping square chamber and the sensing chamber of 10 μl in volume. Since our suggested microfluidic PDMS chip was fabricated with a single SU-8 main mold, the sensing chamber thicknesses of the PDMS chips were supposed to be identical. The thicknesses of ten random PDMS chips have been measured, and the thickness variation was negligible. The vacuum treated PDMS chip pumps up the microalgal sample solution into the sensing chamber as shown in Fig. 3(b).

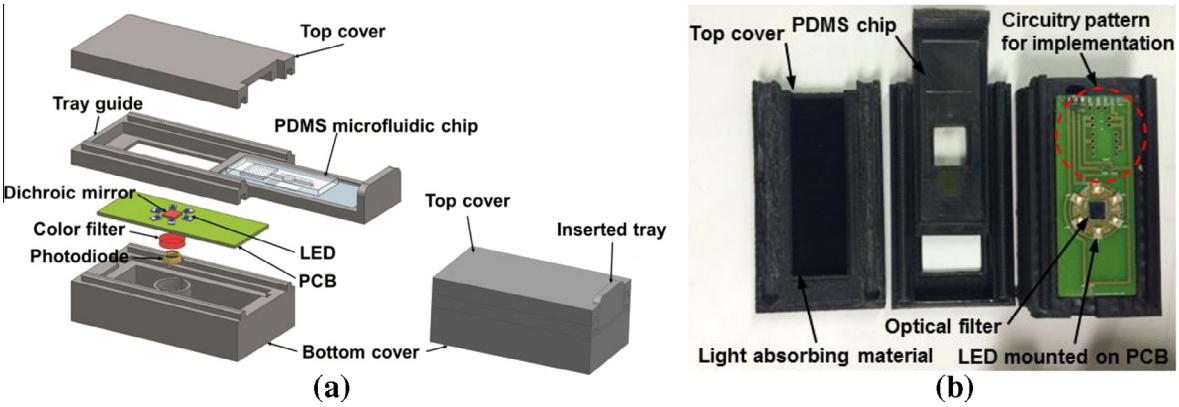

Fig. 2. The designed sensor system: (a) an explode and assembled view of the handheld fluorescent detection system and (b) the 3-D printed sensor housing.

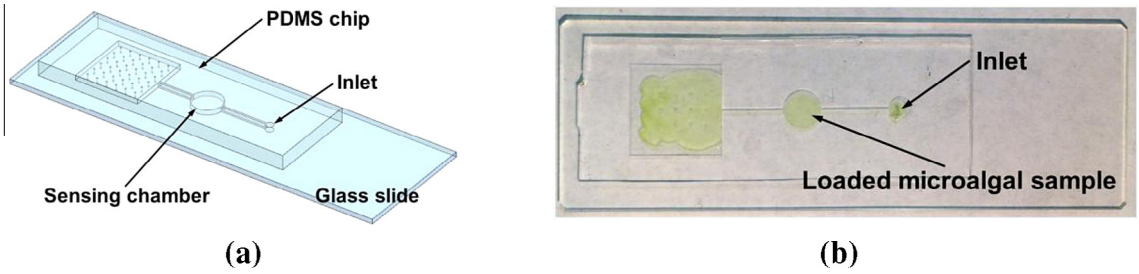

Fig. 3. Sample loading microfluidic chip: (a) schematic of the PDMS microfluidic chip and (b) a photograph of the microalgal sample loaded chip.

The microalgal sample solutions were well shaken to ensure uniform dispersion of microalgae right before loading into the vacuum treated PDMS chip.

2.5. Chlorophyll a fluorescence detection experiments

For measuring the fluorescent signal, the PDMS chips containing different microalgal populations were made separately. The microfluidic PDMS chips were produced with SU-8 standard lithography techniques. After carefully attaching PDMS with a glass slide, fabricated microfluidic PDMS chips were vacuum treated to remove gas molecules trapped in the chips. Prepared microalgal sample (*C. vulgaris*) with different populations were injected into the PDMS microfluidic chips immediately after the vacuum treatment. The 100% concentration of microalgal sample contained 19,000 cells/μl measured by flow cytometer (BD Accuri C6). Lower concentration microalgal samples were obtained by diluting the 100% concentration of microalgal sample with culture media. Also, predetermined microalgal sample was mixed with turbid water studying the effects of suspended particle in water. The slide glass with a PDMS microfluidic chip was deployed on the 3D printed tray to insert easily into the sensor system. Six excitation LEDs were connected in series and driven with 50 mA forward current when microfluidic PDMS chip was inserted into the device. The photodiode was reverse biased with −20 V to acquire linear response to the fluorescent input light, and it was connected to a picoammeter (Keithley 6485, USA) to measure and record the photocurrent. The picoammeter collected a total of 300 sampling points with second interval for each microfluidic PDMS chips.

3. Results and discussion

3.1. Characteristics of the components

The blue LED from Philips Lumileds was selected due to the relatively cheap price and narrow wavelength range (447 nm ± 10 nm). Moreover, since it is a surface mountable LED, heat is easily dissipated through the PCB thermal pad, thus experiencing no light intensity drop while in operation.

The emission spectrum of the blue LEDs was measured using a portable spectrometer (Ocean Optics, USA). The result shows that the blue LED has its peak at 443 nm wavelength with 13.2 μW/cm^2 light intensity. Transmittance of a dichroic mirror filter and a color filter was also measured using a spectrophotometer (Hach Company, USA). The dichroic mirror has a cut-off wavelength at 674 nm and the transmittance at 680 nm was 95%. The color filter has a cut-off wavelength at 645 nm and the transmittance at 680 nm was 65%. The color filter is 3 mm in thickness and the dichroic mirror is only 1 mm in thickness showing better transmittance in the bandpass range. The dichroic filter selectively transmits or reflects the light based on the wavelength of the light that is angle-dependent. The incident angle of the reflected excitation light is not always normal and as a result, a small amount of blue light passes through the dichroic filter without being reflected. On the other hand, the color filter is less sensitive to the incident light angle, but due to the dye material on the glass, it shows autofluorescence contributing to background noise [30]. Therefore, a two-filter system can minimize the background noise condition during the operation (see Fig. 4).

3.2. Fluorescence measurement of microalgal sample

We analyzed the performance of our fluorescent sensor by measuring the microalgal cell concentration. To test the microalgae fluorescence, six different concentrations were made with different ratios of the stock microalgal sample (*C. vulgaris*) with a cell concentration of 38,000 cells/μl read from a BD Accuri C6 Flow Cytometer. The test for microalgae fluorescence with turbid water will add an extra dilution; therefore the microalgae only solutions were prepared to keep the same cell concentration as the samples mixed with turbid water. The following is the ratio of microalgae and water (algae volume:water volume) along with the approximate

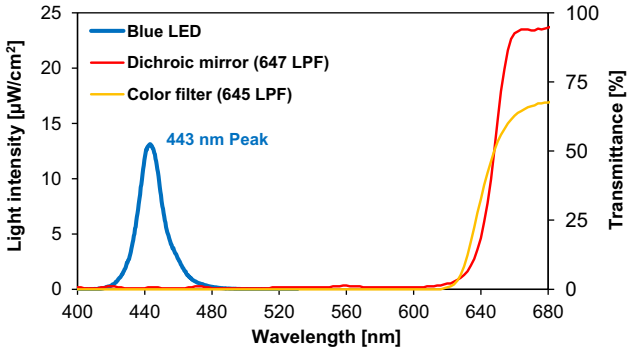

Fig. 4. Measured spectra of the excitation LED and the optical filters used in the fluorescent detection system.

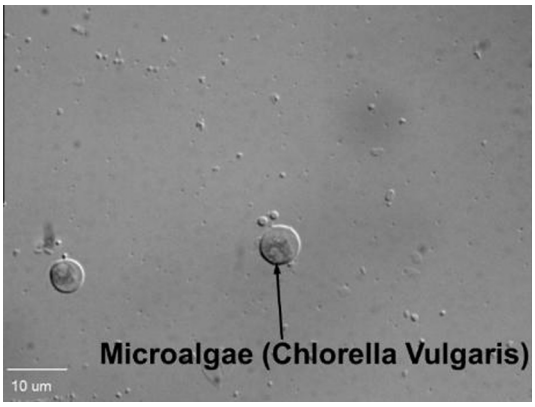

Fig. 5. Microscopic image of microalgae (*C. vulgaris*) sample.

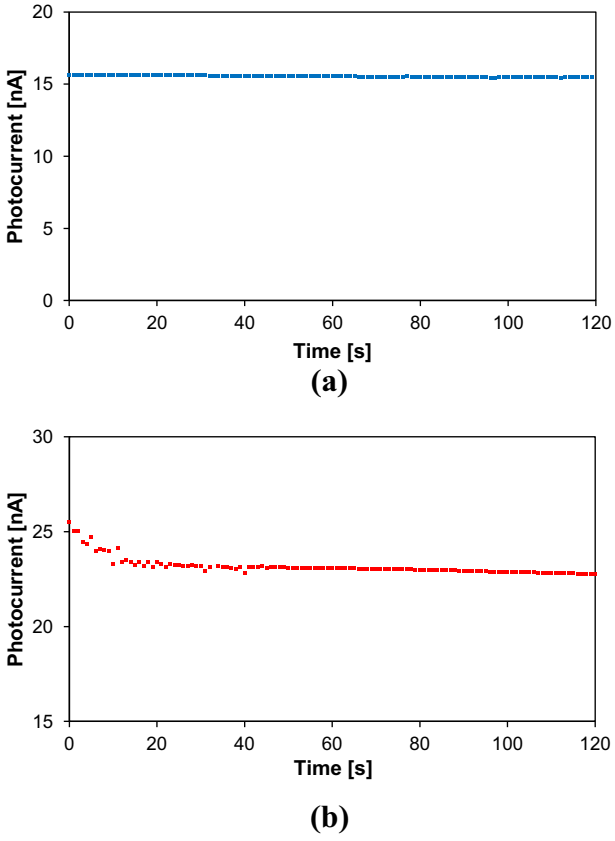

Fig. 6. Measured photocurrent values: (a) back ground noise with a water only sample due to the leakage of excitation light and (b) fluorescent light signal over time with a microalgal sample (19,000 cell/μl). The fluorescent light intensity decreases over time due to the quenching effect.

cell concentration (cells/μl): 1:9 (3800 cells/μl), 1:4 (7600 cells/μl), 3:7 (11,400 cells/μl), 2:3 (15,200 cells/μl), 1:1 (19,000 cells/μl). The microfluidic chips with microalgal sample solutions were kept in the dark for more than 20 min before the test as a means to maximally oxidize the primary quinone electron acceptor of Photosystem II and open Photosystem II reaction center [16]. Then, the microfluidic chip was loaded into the sensor and the excitation light was turned on. Fig. 5 shows a microscope pictures of the microalgae cells tested.

Fig. 6(a) shows that the excitation light creates background noise signal due to the light leakage. Some reflected LED light rays penetrate the optical filters even though most of them were filtered out. However, we also confirmed that the excitation light intensity was stable and not degraded over the duration of measurement. Since the LED light does not require a time for stabilization, it can reduce the power consumption and preparation time before the measurement. The photocurrent signal data was recorded and saved with a 6485 picoammeter (Keithley, USA). Fig. 6(b) shows that fluorescent light intensity from a microalgal sample (19,000 cells/μl) decreases over time due to the photochemical quenching effect [16]. Therefore, the LED was allowed to run for 120 s and photocurrent was measured every 1 s interval. When the excitation light was on, the photocurrent quenching occurred for 20 s and the signal was stabilized. Microfluidic chips for testing microalgal samples at varying concentrations were prepared and corresponding stabilized photocurrent values were correlated with the cell count values obtained from the BD Accuri C6 Flow Cytometer. Fig. 7 is the test result of the fluorescent sensor with different microalgae concentration measurements. The result shows that fluorescent sensor has a linear response compared to

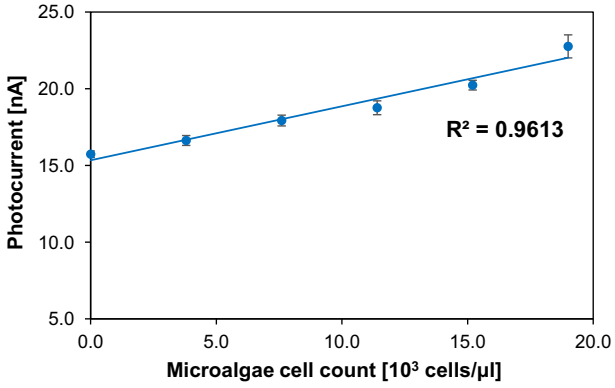

Fig. 7. Fluorescent photocurrent vs. microalgae concentration. The error bars indicate one standard deviation with a sample number of $n = 3$.

Table 1
Soil composition of the turbid water.

Property	Units	
Sand content	%	73.2
Silt	%	16.3
Clay	%	10.5
Median grain size diameter	mm	0.2

the flow cytometer cell counting measurement. The critical issue is the excessive noise from the light source. The highest concentration measurement data of the developed sensor was found to be

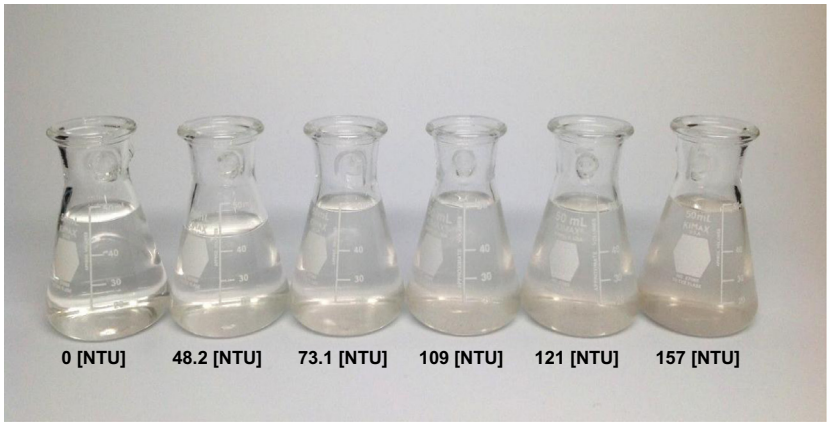

Fig. 8. An image of water samples with varying turbidity levels.

Table 2
Photocurrent measurement results of the microalgal samples mixed with different turbidity water. Photocurrent values of the microalgal sample with same cell count are constant regardless of the turbidity level of mixed water.

Measured photocurrent [nA]									
Turbidity of the mixed water [NTU]	0	23.5	48.2	73.1	109	121	157	Avg.	SD (σ)
0 cells/μl	15.7	15.7	15.9	15.9	16.3	16.0	15.7	15.9	0.206
3800 cells/μl	16.6	17.4	16.9	17.2	17.2	17.7	16.1	17.0	0.521
11,400 cells/μl	18.8	19.2	19.1	18.8	18.6	18.8	19.0	18.9	0.215
19,000 cells/μl	22.8	21.5	23.6	22.0	21.1	22.5	22.4	22.3	0.826

19,000 cells/μl according to our experimental results, which is higher than the harvesting concentration of the microalgal products from the HISTAR system (15,000 cells/μl) [24,25].

3.3. Fluorescence measurement of turbid microalgal sample

The culture media of outdoor raceway cultivation system for microalgae may include various suspended particles such as dirt. Moreover, lake and pond water will contain suspended particles with microalgae. The suspended particles in turbid water are one of the possible error sources for the microalgal sensors [28]. The fluorescent signal from microalgal cell can be attenuated by the scattering effect.

To investigate the influence of turbid water mixed with a microalgal sample solution, different mixtures of turbid water and microalgal sample were prepared. Six stock cultures of turbid water at different turbidity levels were prepared and measured using a 2100P ISO HACH Turbidimeter and BD Accuri C6 Flow Cytometer. The soil used to make turbid water is similar in size and properties as that found in samples from unlined algal ponds in Louisiana (Table 1). Fig. 8 shows water sample at the different turbidity levels, from 0 to 157 NTU or nephelometric turbidity units.

Table 2 shows the test result of the fluorescent sensor with different microalgae concentration mixed with turbid water. The result confirms that the fluorescent sensor has a linear response compared to the flow cytometer cell counting measurement regardless of the turbidity level of the mixed water.

Table 2 validates that the turbidity level of the water mixed with microalgae sample was not affected by the photocurrent signal obtained from the photodetector. This experimental result demonstrates that the fluorescent sensor has an advantage over turbidity sensor in terms of selectivity, especially in detecting microalgal sample with contaminants.

4. Discussion and conclusions

There are a few improvements that could make the fluorescent sensor fully portable, multi-detectable, and more accurate. One issue is the low signal-to-noise ratio due to the excessive reflected light from the excitation LED light. Since the light source and the detector are closely located, the excessive reflected light generates a background noise to the photodetector. A light absorbing sheet (Thorlabs, USA) attached on the inner sensor wall removed most of the noise, but still showed 13 nA background photocurrent noise (dark noise = 0.1 nA). We believe that further reduction of noise can be achieved by focusing the excitation light to the sample chamber. The current excitation LED light has a large beam angle (120°), thus generating excessive reflected light noise, therefore a focused beam light can reduce the reflection in a sensor jig wall.

Multiple integrated light systems with an improved sensitivity feature will make it possible to differentiate different species in the sample solution. The sensitivity of our microalgae fluorescent sensor can be improved by replacing with a more sensitive photodetector. Moreover, there are abundance of other organisms and molecules that can be detected using the developed fluorescent sensor. A detection circuit with display and battery can be added to the developed sensor to make it a fully portable sensor system.

In summary, we have developed a portable and low-cost fluorescent sensor for on-site detection of microalgae with a disposable PDMS microfluidic chip. A 448 nm wavelength LED was selected to excite the microalgae that emit 680 nm wavelength fluorescent light. A photodiode with a 645 nm long-pass optical color filter and a dichroic mirror was mounted below the PDMS microfluidic chamber to detect the fluorescent light signal from the samples. The experimental results have confirmed that the fluorescent sensor has a linear response comparable to the flow cytometry cell counting measurement. Selective microalgae detection to a turbid sample was also demonstrated. The fluorescent sig-

nal was independent of the turbidity level of the sample and the result from microalgal concentration measurements in different levels of turbid samples showed a linear response comparable to the cell counting measurement as well. Future improvements include continuous noise reduction and integration of a detection circuit system to enable the on-site detection.

Acknowledgements

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