# Prototype Microfluidic System for Fluorescence-Based Chemical Sensing

Pattareeya Kittidachachan<sup>1</sup>, Suparat Rujihan<sup>1</sup> and Badin Damrongsak<sup>2\*</sup>

<sup>1</sup>Department of Physics, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand <sup>2</sup>Engineering Physics Research Group, Department of Physics, Faculty of Science, Silpakorn University, Nakorn Pathom 73000, Thailand

\*Corresponding author. E-mail:badin@su.ac.th

## ABSTRACT

A prototype microfluidic system for fluorescence detection is presented in this paper. The system consists of an excitation light source, a polymer-based microfluidic channel and an optical detector. Experiments are conducted to demonstrate use of the developed system for fluorescence detection in chemical applications. Three fluorescent dyes – Rhodamine 6G, Coumarin 153 and DCM – with different concentrations ranging from 100 nM to 5 mM are used as the analyte in the experiments. Relationship between the photo-response of the microfluidic system and concentration of fluorescent dyes is investigated. The results are compared to that obtained from a bulk system where the analyte under test is dispensed into a standard-size cuvette. In the bulk system, we found a decrease in the fluorescence signal when dye concentration is higher than 10-5 M for Rhodamine 6G and 10-4 M for Coumarin 153 and DCM. This is probably due to the re-absorption and self-quenching phenomena, which result in low quantum yield of fluorescent dyes at high concentration. However, this problem is not found in microfluidic systems with a low detection volume. In addition, the relationship between the photo-response of the system corresponding to different dye concentrations was modeled based on Beer Lambert's law. The measured results agreed will with the theoretical model.

Keywords: Fluorescence detection, Lab-on-a-chip, Chemical sensor, Optical detection system

#### **INTRODUCTION**

Fluorescence detection is a reliable and highly sensitive technique for measuring toxic heavy metal contamination (e.g., mercury, cadmium and lead) in the ambient environment (Basabe-Desmonts et al., 2007; Demchenko, 2009; Liu et al., 2010). Commercially available fluorescence systems are typically bulky and expensive, so inconvenient for on-site measurement, which requires small devices. For this reason, considerable research has focused on developing compact and low-cost fluorescence detection systems (Kou et al., 2009; Banerjee

et al., 2010; Zhang et al., 2011). In recent decades, because of their portability and miniaturization, which enables them to work with small amounts of volume/ sample, microfluidic systems have attracted interest in a variety of fields, including biology, chemistry and physics. The fabrication of microfluidic devices is relatively simple and cheap; thus, making such devices an ideal choice as portable sensors to detect toxic heavy metals.

In this paper, we present the development and characterization of a prototype microfluidic system for fluorescence detection. The diagram of the prototype microfluidic system is shown in Figure 1. A high power LED is used as a main light source, instead of a tungsten lamp or a laser, thus making the system more compact and inexpensive. This system is similar to previous work developed by Banerjee et al. (2010), in which two polarizers were used to remove the unwanted light emitted by the light source. However, with such a system, the fluorescence signal was partly absorbed by a polarizer. Instead, in this study, a long-pass filter is used to eliminate only the unwanted light from the LED light source that has overlapped with the fluorescence spectrum of the dyes.



Figure 1. Diagram of a prototype microfluidic system for fluorescence detection.

In addition, a theoretical model is developed to investigate the relationship between the photo-response of the microfluidic system and concentration of fluorescent dyes. Fabrication of a microfluidic channel is described. Experiments are performed and results are then compared with the developed theoretical model.

#### THEORETICAL BACKGROUND

A theoretical model based on Beer Lambert's law is developed in order to calculate the output of a prototype microfluidic system corresponding to different dye concentrations. First, when the light with the excitation power  $P_0$  travels through a microchannel containing dye solution, the transmitted power  $P_T$  can be expressed using Beer Lambert's law (Banerjee et al., 2010) as:

$$P_T = P_0 e^{-\alpha(\lambda)x} \tag{1}$$

where x is the optical path length through the dye solution and  $\alpha(\lambda)$  is the absorption coefficient.

The power that is absorbed by fluorescence dye can then be calculated using Equation (2):

$$P_A = P_0 - P_T = P_0 \left( 1 - e^{-\alpha(\lambda)x} \right)$$
<sup>(2)</sup>

where  $P_A$  is the absorbed power.

When a dye is illuminated by the excitation light, it emits photons at a longer wavelength with a power  $P_{F}$ . The amount of emitted fluorescence power is proportional to the absorbed power and the fluorescence quantum yield  $\phi_{F}$ , which can be given by:

$$P_F = \phi_F P_A = \phi_F P_0 \left( 1 - e^{-\alpha(\lambda)x} \right).$$
(3)

The fluorescence quantum yield is the ratio of the amount of emitted light over that of absorbed light.

In our microfluidic system, the emission light is observed through a long pass filter that suppresses the excitation wavelength. Therefore, only some emitted fluorescence can reach a photodetector. The output photocurrent  $I_{photo}$  is then proportional to the responsivity of the photodetector itself, and the amount of emitted fluorescence reaching the photodetector, which can be given as:

$$I_{photo} = R(\lambda) T_{filter} \phi_{CE} P_F \tag{4}$$

where  $R(\lambda)$  is the wavelength dependent responsivity of the photodetector,  $T_{filter}$  is the transmittance of the long pass filter and  $\phi_{CE}$  is the collection efficiency, which is the ratio of the amount of collected fluorescence over total emission fluorescence.

Replacing the fluorescence-emitted power from Equation (3) into (4), we have:  $(a_1, a_2)$ 

$$I_{photo} = R(\lambda) T_{filter} \phi_{CE} \phi_F P_0 \left( 1 - e^{-\alpha(\lambda)x} \right)$$
(5)

For the sake of simplification, some term is combined into a single lumped parameter K, called a K-factor. The K-factor depends on the responsivity of the photodetector, the filter transmittance, the collection efficiency of the system, the fluorescence quantum yield and the excitation power:

$$I_{photo} = K \left( 1 - e^{-\alpha(\lambda)x} \right)$$
(6)

where  $K = R(\lambda)T_{filter}P_0\phi_{CE}\phi_F$ .

In chemistry, the absorption coefficient is proportional to the molar absorptivity  $\varepsilon(\lambda)$  and the concentration *c* of a chemical species as:

$$\alpha(\lambda) = \varepsilon(\lambda)c \tag{7}$$

Hence, the output photocurrent as a function of concentration can be written as:

$$I_{photo}\left(c\right) = K\left(1 - e^{-\varepsilon(\lambda)cx}\right)$$
(8)

Equation (8) is used for fitting experimental data for all experiments in this paper.

## MATERIALS AND METHODS

## Fabrication of microfluidic channels

Microfluidic channels used in this experiment were fabricated in house using the process flow described in Rujihan et al. (2012). First, the master mold of the desired pattern was fabricated using printed circuit board technology. The next step, called replica molding, used poly-dimethysiloxane (PDMS) as a starting material. This PDMS part was used to create microfluidic devices. The last step is PDMS-glass bonding. Before bonding, holes were drilled manually in PDMS for fluidic connections. Surface treatment using oxygen plasma was used to clean both PDMS and the glass slide. Immediately after plasma treatment, PDMS is placed into contact with the glass slide to complete the bonding.

#### Experimental setup

Experiments were conducted to study the relationship between the photoresponse of the microfluidic system and concentration of fluorescent dyes. Three fluorescent dyes – Coumarin 153, DCM and Rhodamine 6G (Rh6G) – with different concentrations ranging from 100 nM to 1 mM were used as the analyte in the experiments. In this study, a high-power LED was used as the excitation light source. It was chosen in such a way that its peak wavelength and the absorption spectra of the fluorescence dyes overlapped. Therefore, a UV LED with a peak wavelength of 396 nm was used as the excitation light for Coumarin 153, a blue LED with a peak wavelength of 451 nm for DCM dye and a green LED with a peak wavelength of 396 nm for Rh6G. Emission fluorescence was then observed by (1) a spectrophotometer (Avantes) in order to study the emitted spectra of fluorescence dyes and (2) a silicon photodetector (Roithner Laser) in order to study the corresponding photocurrent as a function of dye concentration.

Figures 2 and 3 show the diagram of the experimental setup for fluorescence spectroscopy measurement when the sample is contained in a standard-size cuvette and a microfluidic channel, respectively. The investigation of the photocurrent in response to the variation of dye concentration was carried out using the experimental setup shown in Figure 1.



Figure 2. Diagram of the experimental setup for fluorescence spectroscopy measurement when sample is in a cuvette holder.



Figure 3. Diagram of the experimental setup for fluorescence spectroscopy measurement with a microfluidic channel.

# **RESULTS AND DISCUSSION**

The emission spectra of fluorescence dyes (Coumarin 153, DCM and Rh6G) corresponding to different dye concentrations varying from  $10^{-7}$  to  $10^{-3}$  M are shown in Figures 3, 4 and 5, respectively. In the bulk system, we found a decrease in the fluorescence signal when dye concentration is about 1mM. This

is probably due to the re-absorption and self-quenching phenomena, which result in low quantum yield of fluorescent dyes at high concentration. However, this problem is not found in microfluidic systems with a low detection volume; thus, giving it a dynamic range greater than the bulk system.



Figure 4. Fluorescence spectra of Coumarin 153 dyes in ethanol solution with different concentrations. The solution is contained in (a) a cuvette holder and (b) a microfluidic channel.



Figure 5. Fluorescence spectra of DCM dyes in ethanol solution with different concentrations The solution is contained in (a) a cuvette holder and (b) a microfluidic channel.



Figure 6. Fluorescence spectra of Rh6G dyes in ethanol solution with different concentrations. The solution is contained in (a) a cuvette holder and (b) a microfluidic channel.



**Figure 7.** Experimental results and theoretical model of the photocurrent corresponding to different dye concentrations.

The relationship between the output (photocurrent) of the prototype microfluidic system and various dye concentrations was investigated using the experimental setup shown in Figure 1. The experimental results for all three fluorescence dyes are shown in Figure 7. Using Equation (8), a theoretical model was fit to the experimental data. The optical path *x* is measured from the height of the microchannel, which is equal to 70  $\mu$ m. The molar absorptivity  $\varepsilon(\lambda)$  was taken from the standard value (PhotochemCAD package, version 2.1a) at the excitation wavelength. K-parameters were chosen in such a way that the R<sup>2</sup> value of the trend line was very close to 1. Fit parameters are shown in Table 1.

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Dyes	K	ε (x 10 <sup>5</sup> )	<b>R</b> <sup>2</sup>	
Coumarin 153	8.00	1.16	0.89	
DCM	10.20	2.00	0.81	
Rh6G	0.24	4.20	0.91	

 Table 1. Fit parameters for each fluorescence dye.

#### CONCLUSION

A prototype microfluidic system for fluorescence-based heavy metal detection in the ambient environment was developed and characterized. The results obtained from the prototype microfluidic system show a higher dynamic range than that of the bulk system. This is due to the re-absorption and self-quenching found in the bulk system. In addition, the relationship between the photo-response of the system corresponding to different dye concentrations was modeled based on Beer Lambert's law. The measured results agreed well with the theoretical model.

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