Fluorescence detection system and laminate-based disposable microfluidic chip

Rudi Irawan[1], Swee Chuan Tjin[2], Dianwen Zhang[1], and Xiaoqin Fang[1]

[1] *Biomedical Engineering Research Centre, Nanyang Technological University, Singapore 637553*

[2] *Photonic Research Centre, School of Electrical and Electronic Engineering, Nanyang Technological University, Singapore 639798*

Development of a compact fluorescence point-of-care system (POCS) is challenging. A prototype of compact fluorescence detection system and disposable microfluidic chip reported in this letter used a blue LED, 600 μm fiber, CCD detector, mylar and polymethyl-methacrylate (PMMA), and fluorescein solution as an excitation source, light-guide, detector, microfluidic chip substrates and samples, respectively. The results show the system is able to detect 0.01–1000 μg/l fluorescein in tris buffered saline pH 8.0 and gives linear response. This prototype can be used as a platform to develop a simple and compact multichannel-fluorescence point-of-care bioassay system with an inexpensive and disposable microfluidic chip.

OCIS codes: 170.6280, 120.1880, 160.5470.

Point-of-care system (POCS) has revolutionized the continuous care process of patient by providing laboratory results efficiently and fast at the patient's bedside. The performance of quantitative immunoassays is today largely restricted to centralized laboratories because of the need for long assay times, and relatively bulky, complex and expensive equipment, as well as highly trained operator. Most of immunoassays remain within the walls of large centralized laboratories, far from the patients whose samples are measured. If a wider range of immunoassays could be run simpler, more inexpensively and at the point of care or in the home health care, the health of millions of patients could be improved annually. To achieve this objective, a simple, compact, smart, robust, and inexpensive device providing high quality results is required.

Because of some advantages of sensitivity, simplicity and immunity to electromagnetic wave interferences, optical biosensors represent one of the major families of biosensors to have been exploited for immunoassay applications[1]. Among of optical biosensor techniques, fluorescence-based sensors are the most highly developed due to their high sensitivity, versatility, accuracy and fairly good selectivity[2]. Fluorescence method is also very suitable for miniaturization[3].

The trend to miniaturization has been discussed in several publications[4,5]. In clinical practices it is often faced on the problem of very small volume of sample. These facts immediately bring the term of miniaturization into the realm of microfluidics[6,7]. Microfluidic technology is ideal for handling costly and difficult-to-obtain samples and reagents, because it requires very small volume of samples, in order of μL or even nL. The miniaturization of chemical reactions to the microfluidic system would lead to the reduction of reagent volume, the decrease in analysis time, and the capability of carrying on simultaneous analyses of different analyses. In this letter, integration of fluorescence detection system and disposable microfluidic chip has been developed and tested.

Besides the commonly used glass and silica wafers, polymer-based materials, such as polydimethylsiloxene (PDMS), polymethyl-methacrylate (PMMA) and polycarbonate[5,8−12] have been introduced as microfluidic materials recently. Thin laminate sheets, such as mylar and PMMA, have very good seal characteristic, non-porous solid, so they do not absorb the biomolecule sample[10]. Microfluidic channels based on laminate can be fabricated easily through molding, embossing and casting processes, while other materials may need to use the time-consuming photomask generation, photolithography, and etching processes which hamper the rapid turnaround of new design. In this experiment, three channels 250 μm in width were machined by the ablation process when the CO_2 laser beam was focused on a 50-μm-thick Mylar sheet, producing a channel cross-section 50×250 (μm). Before fabrications, the channel patterns were drawn using graphical computer software, and the patterns were transferred to the direct-write CO_2 laser cutting machine.

Mylar-D has the potential to be used as the substrate of a disposable microfluidic chip as it has very good optical properties, such as transparency in the visible wavelength, inexpensive and widely available. The use of direct-write laser micromachining on mylar to fabricate microfluidic chip has the potential for fast flexible prototyping and reproducible mass production. Various complex channels on the material can be easily generated and put to work in hours instead of weeks. However, mylar has relatively high autofluorescence intensity if excited in UV, blue, and green regions[13]. In order to avoid high autofluorescence background from the substrates, PMMA sheets that have low autofluorescence emissions were used at the bottom and the top microfluidic channels as shown in Fig. 1. The mylar and PMMA sheets were held together using 3M501 pressure sensitive adhesive, and pressed between two plates at temperature 40° overnight.

LED is an excellent source for a compact fluorescence system, because it is small, inexpensive, stable, and robust. The LED requires a low driving power and display excellent stability, i.e., as good as the power supply. Here, 470-nm blue LED was chosen as an excitation source, since it is the wavelength near to the absorption peak of fluorescein around 490 nm. The fluorescein diluted inside tris buffered saline pH 8.0 was used as a sample for testing of the system. A drawback of using LED is that its beam diverges, and results in circular o-rings when focused. However, these circular o-ring modes disappear if

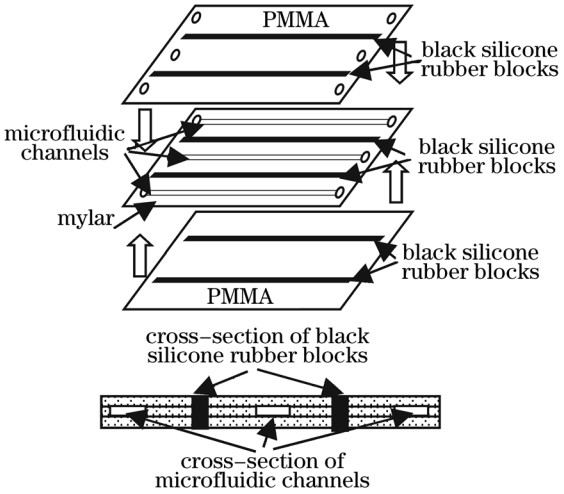

Fig. 1. Microfluidic system and its cross-section.

the light from LED is transmitted through a fiber before focused. The light beam from the LED was filtered by a 470 ± 5 nm band-pass interference filter and focused onto the 600-μm optical fiber. The light from this fiber was focused vertically by a focusing lens system onto the samples inside the microchannels. The fluorescence emission from the samples were collected by the same lens system and focused onto the same fiber. The fiber and focusing lens system were mounted on the micromanipulators, so that they could be positioned accordingly. Then the fluorescence emission was transmitted by the fiber toward the CCD detector, through a mirror and 500-nm long-pass filter. The use of optical filters made it possible to have good frequency separation between the emission and the excitation light. The low signals from the detector were amplified and interfaced to a personal computer for reading. The sketch of the fluorescence detection system and microfluidic system are shown in Fig. 2.

The function of the optical fiber is not only to transmit the excitation and fluorescence emission light, but the fiber together with a 0.5 mm diameter pinhole in front of the detector also rejects the stray light entering the detector. The intensity of fluorescence emission from the samples in the microfluidic channels is usually very weak, so it is very important to avoid the unnecessary losses of collected fluorescence. Previously, fibers with 1×2 fiber coupler (Y coupler) were considered to transmit the excitation and the fluorescence light. However, this type of fiber configuration causes the collected fluorescence light which is split into two parts, that is, one is transmitted toward the detector side and the other is transmitted toward the excitation source side. The one transmitted toward the excitation source side is the loss in this case. In addition, the coupler itself may also cause the loss. To avoid this type of loss, a single type of fiber was used both for excitation and fluorescence light transmissions. In order to accommodate both excitation source system and detection system at one side of fiber, the excitation beam from the LED was focused into the fiber at angle 20° from the optical fiber axis, and the fluorescence light was collected at the optical fiber axis as depicted in Fig. 2. This particular geometrical configuration of the optical setup made it also possible to eliminate the excitation light transmitted and/or reflected toward the detector.

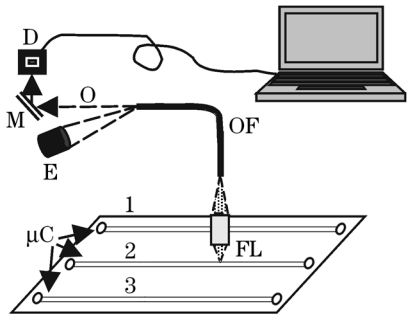

Fig. 2. The geometrical setup of the experimental system. μC: microfluidic chip and channels; FL: focusing lens system; OF: optical fiber; E: excitation source system; O: objective lens; M: mirror; D: detector; and 1, 2, and 3: channel numbers. The distances between the channels are 2 mm.

The sensitivity of the integrated device was evaluated by filling the microchannels with known concentrations of fluorescein in tris buffered saline pH 8.0. The samples were stored inside the protected syringes that were connected to a microfluidic pump. During the measurement, the samples were continuously pumped through the channel at a constant speed, so that the photobleaching effect was minimized. Between the measurements, the channels were cleaned thoroughly, flushed with buffer, and the process was repeated. The fluorescence intensities of various fluorescein concentrations were corrected with respect to the background fluorescence, which was the fluorescence of the channel loaded by buffer only. Figure 3 shows plot of the fluorescein concentrations against the normalized fluorescence intensities. It shows that the system is sensitive. It was easily able to detect 0.01-μg/l fluorescein in tris buffered saline. The signal obtained in this concentration was still significantly above the noise level, more than fifty times higher. This result indicates that the sensitivity of the system is adequate to detect low concentration of fluorescently-tagged analytes and it is in linear response range.

To evaluate the consistency of the results for a multichannels system, the measurements were tested for three different channels in one microfluidic chip. The specifications of the channels, samples, and detection system were similar. Figure 4 shows that the fluorescence intensities detected from three different channels were very similar.

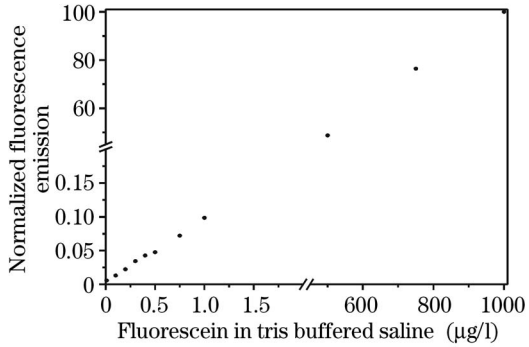

Fig. 3. Concentration of fluorescein in tris buffered saline versus fluorescein intensity.

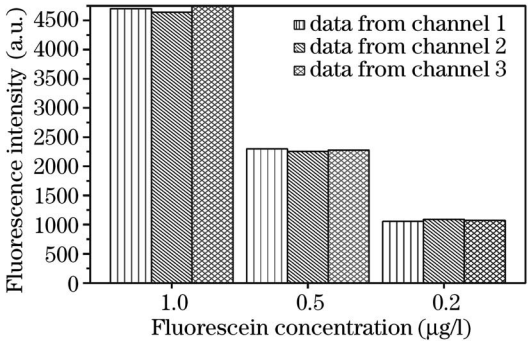

Fig. 4. Fluorescence signals collected from three different microchannels in one microfluidic chip.

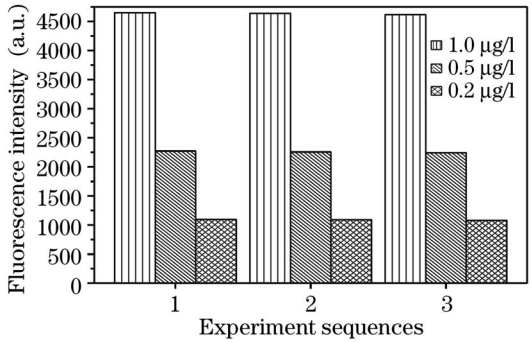

Fig. 5. Reproducible results obtained from three repetitions of fluorescence measurements from channel 2.

Another requirement of a sensor is reproducibility. Figure 5 shows that the results are highly reproducible with the statistical errors are less than 0.5 %. It means there was no stain from previous samples left in the channel after cleaning.

A multichannel-fluorescence system may be required for simultaneous analysis of different analyses. Since Mylar and PMMA are transparent in visible region and have refractive indices higher than the refractive index of air, they can behave as a waveguide for fluorescence emission and excitation beam, and cause cross-talks between the adjacent channels[14]. To avoid the cross-talks, 100 μm in width of black silicone rubbers from Bondo/Mar-Hyde Corp Atlanta were placed between the channels as shown in Fig. 1, and then the cross-talk tests were conducted. The tests were conducted by filling three microchannels with 1 mg/l of fluorescein. The sample inside the channel 2 in Fig. 2 was illuminated by focusing the excitation beam directly into the channel, and the fluorescence emissions from the channels 1 and 3 were measured. It was observed there was no fluorescence detected in the channels 1 and 3. It indicates the microfluidic chip construction used here was able to optically isolate the channels from each other.

In conclusion, this study provides a platform to develop a portable and affordable multichannel-fluorescence system for point-of-care bioassay diagnostic applications. The microfluidic system can be accessed and replaced easily. Fabrication of multi-microchannels in polymeric materials, such as PMMA and Mylar using a direct-write laser micromachining is easy, quick and reproducible. They are also inexpensive, so the microfluidic chip can be treated as disposable. However, Mylar has high autofluorescence background, so that PMMA which has low autofluorescence background should be used at the top and bottom channels. The LED and CCD are an excellent light source and detector for a compact point-of-care system, respectively, because they are compact, simple, inexpensive, stable, and widely available. Transmitting the excitation light and fluorescence emission using a single type of optical fiber enables to avoid the fluorescence loss due to splitting of the light. The system is capable of detecting very low concentration of fluorescein, and the results are consistent, and reproducible. Placing black silicone rubbers between the channels is able to optically isolate the channels from each other, so that the cross-talks due to adjacent channels in a microfluidic chip can be avoided.

This project was supported by the Biomedical Engineering Research Council of Singapore under the Singapore-University of Washington Alliance Programme. R. Irawan's e-mail address is eirawan@ntu.edu.sg.

References

1. P. N. Prasad, *Introduction to Biophotonics* (Wiley Interscience, New Jersey, 2003).
2. J. R. Lakowics, *Principles of Fluorescence Spectroscopy* (Kluwer Academics, New York, 1999).
3. K. R. Diamond, M. S. Patterson, and T. J. Farrell, App. Opt. **42,** 2436 (2003).
4. J. Khandurina and A. Guttman, J. Chromatography **943,** 159 (2002).
5. L. J. Kricka, Clin. Chim. Acta **307,** 219 (2001).
6. T. H. Schulte, R. L. Bardell, and B. H. Weigl, Clin. Chim. Acta **321,** 1 (2002).
7. F. Vinet, P. Chaton, and Y. Fouillet, Microelectronic Eng. **61–62,** 41 (2002).
8. P. Grodzinski, R. H. Liu, B. Chen, J. Blackwell, Y. Liu, D. Rhine, T. Smekal, D. Ganser, C. Romero, H. Yu, T. Chan, and Kroutchinina, Biomedical Microdevices **3:4,** 275 (2001).
9. J. Kameoka, H. G. Craighead, H. Zhang, and J. Henion, Anal. Chem. **73,** 1935 (2001).
10. B. H. Weigl, R. L. Bardell, T. Schulte, F. Battrell, and J. Hayenga, Biomedical Microdevices **3:4,** 267 (2001).
11. H. Klank, J. P. Kutter, and Geschke, Lab on a Chip **2,** 242 (2002).
12. J. Y. Cheng, C. W. Wei, K. H. Hsu, and T. H. Young, Sensors and Actuators B **99,** 186 (2004).
13. R. H. Kenneth, and Y. Paul, Lab on a Chip **3,** 248 (2003).
14. R. Irawan, S. C. Tjin, P. Yager, and D. W. Zhang, Biomedical Microdevices **7,** 205 (2005).