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Miniaturized fluorescence detection system to remove background noise of the incident light using micro mirror and lens

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Abstract

This paper reports a study on the miniaturized fluorescence detection system using reflecting mirror. It is integrated on a microfluidic chip to remove the signal offset and background noise of the incident beam. Previous approaches were integrated optical fiber, inclined incident beam, and split beams by a stop on microlens. However, they have problems of difficult assembly or loss of intensity at center. Therefore, we tried another optical configuration using microlens and mirror, which are patterned on the bottom and top surface of glass microfluidic chip, respectively. The exciting light is focused by the microlens on the bottom mirror and it is reflected out through the incident aperture. Microfluidic chip with microlens and mirror was microfabricated and fluorescence of the microchannel was measured with various size of mirror. The fluorescence signal on photomultiplier tube showed enhanced properties showing smaller offset and less background noise than that of microfluidic chip without micromirror. Theoretical model of intensity propagation of exciting beam and intensity of signal to background ratio was also analyzed to predict the improvement with low lens fluorescence and channel refraction.

Keywords: Fluorescence detection; Microlens; Reflecting mirror

1. Introduction

Micro total analysis system (μTAS) attracts many researchers' attention since it consumes tiny volume of reagent and it analyzes chemical quantity rapidly and effectively [1,2]. Many applications using microfluidic system has been studied such as DNA sequencer, micro PCR, immunoassay, enzymatic assay, and high throughput screening system. Among them portable diagnostic device is the most promising application considering the merit of miniaturization using microfabrication. It enables several analysis procedures (sample preparation, transportation of fluid, separation, mixing, and reaction) to be integrated in a small chip, so called lab-on-a-chip (LOC). Detecting biomolecules in microchannel usually exploits fluorescence since it is highly sensitive, well established and easily implementable with conventional fluorescence microscope. Although electromechanical sensor is also studied widely, its application area is not as wide as fluorescence detection. Hence, portable fluorescence detection system requires miniaturized optical equipment to be useful as a portable device such as laptop or palm-top.

Conventional fluorescence detection system consists of exciting laser, photo-sensor, dichroic mirror and bandpass filter to separate exciting light and emission fluorescence. Primary function of optical detection system is collecting the emitted fluorescence without background noise from exciting light. Since most of equipment space is allocated to optical component for suppressing background, short optical path using microfabrication technique is effective for miniaturized fluorescence detection system. New photo sensor for portable device is studied by Thrush [3] using integrated

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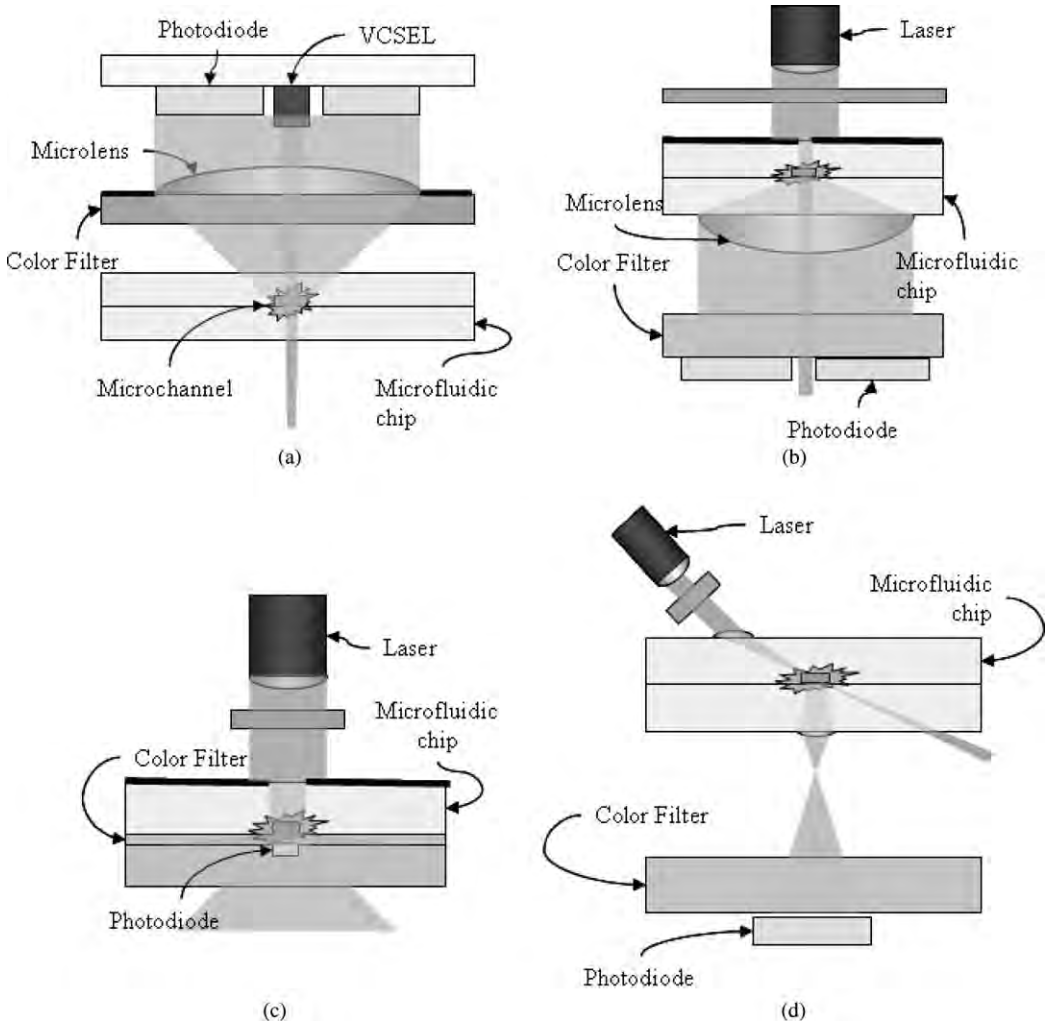

Fig. 1. Several optical schemes for portable fluorescence detection: (a) integrated laser and photodiode: light from VCSEL excites fluorescence in microchannel and the emitted light is collected by the above photodiode; (b) pass through optical layout: exciting beam pass through transparent glass aperture surrounded by the photodiode; (c) direct sensing scheme: exciting laser beam is filtered only by the color filter integrated in chip; (d) inclined beam setup: inclined exciting beam passes through microfluidic channel.

vertical-cavity surface-emitting laser (VCSEL) and photodiode with DBRs (distributed Bragg reflectors) filter as shown in Fig. 1(a). Kamei et al. [4] detected separated DNA and amino acid using amorphous silicon photodiode with pinhole where the exciting beam passes through (Fig. 1(b)). Namasivayam et al. [5] fabricated on-chip photodiode shown in Fig. 1(c) for miniaturized genetic analysis system. However, new photodiode system needs complex fabrication processes costing a lot. Another approach using commercial photo sensor and filter is modifying the optical layout with microlens. Off-axis illumination was suggested by Roulet [6], which needs auxiliary optical jig to focus the inclined laser beam and eventually occupies quite a big space as shown in Fig. 1(d). They also tested central stop at lens and exciting filter, but the exciting efficiency was not good due to blocking central beam of exciting laser.

Hence, we placed a micro mirror at the bottom of glass chip, which reflects the exciting laser and effective in exciting fluorescence (Fig. 2). When microlens focuses incident beam into a point at bottom, no background from the exciting beam occurs theoretically, though micro mirror causes loss of fluorescence. The chip separates the exciting beam and emitted fluorescence effectively and reduces the height of optical detection system. The fabrication of suggested configuration is relatively easier and less expensive than custom-designed photodiode such as photodiode with pinhole and on-chip photodiode [4,5]. It needs no external focusing work like inclined beam set-up [6] since the aligning with lens and mirror is done by precise mask aligning process. This miniaturized optical detection chip could realize portable fluorescence detection system for emergency or environment monitoring if light source and photosensor is installed in cartridge type reader. This paper will discuss the fabrication of microlens and mirror on glass microfluidic chip and analyze the effect of mirror diameter on the background noise due to the incident beam by both theoretical modeling and experiments.

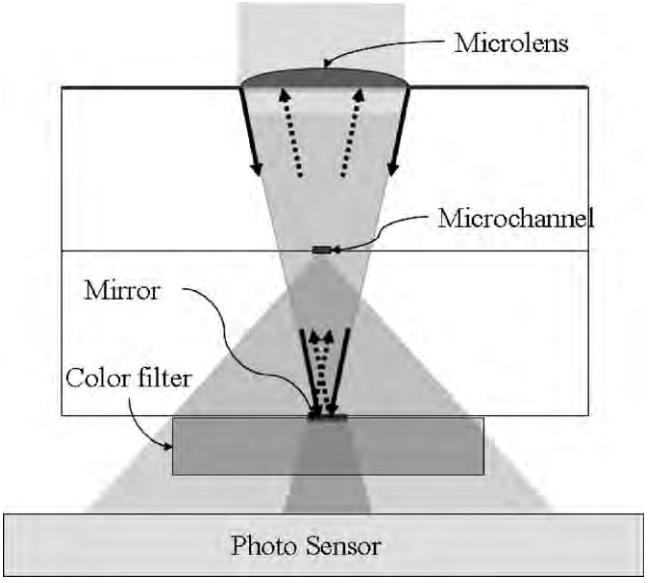

Fig. 2. Central stop scheme: exciting beam is focused on the bottom of chip by microlens and reflected by micro mirror.

2. Materials and methods

2.1. Design of microlens

Microfluidic chip was designed for capillary electrophoresis of DNA. The microchannel was 100 μm wide and 30 μm deep. As the thickness of Pyrex wafer is 500 μm, it is required the nominal focal length of microlens be 1000 μm for exact focusing. However, the tolerance of microlens fabrication demands the focal length be more than the nominal length, because microlens whose focal length is less than 1000 μm cannot reflect out exciting laser through aperture as shown in Fig. 3. If lens height varies by 5 μm, the focal length changes by 150 μm more or less from Eq. (1), which describes the vertex focal length f of a plano-convex refractive photoresist microlens in glass chip [7].

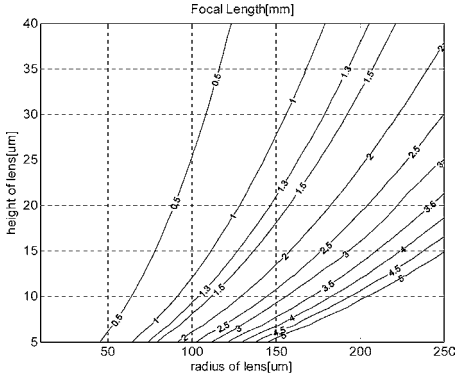

Fig. 4. Contour plot of focal length as a function of radius and height of microlens.

$$f(r,h) = \frac{r^2 + h^2}{2h(n_{\mathrm{PR}} - 1)} n_{\mathrm{glass}}, \quad (1)$$

where r is the radius, h the height and n_{PR} and n_{glass} are refractive indices of photoresist and glass respectively. We conservatively determine the nominal focal length as 1300 μm, the radius as 150 μm, and the height as 21 μm from the contour plot of focal length (Fig. 4). Ray trace analysis using simulation package (Zemax) expected the diameter of focused beam as 75 μm at the bottom of chip. The radius of mirror was designed to vary from 68 to 277 μm to investigate the effect of fluorescence loss by mirror size.

2.2. Fabrication of microfluidic chip and microlens

Standard photolithographic techniques were used to manufacture glass chips [8]. Chrome and gold was deposited on a Corning 7740 Pyrex glass substrate (Corning Co.) using electron beam evaporator for a mask against HF etching. AZ1512 Photoresist (Clariant Corp.) was then spin-coated, soft-baked, and selectively exposed to ultraviolet light. The

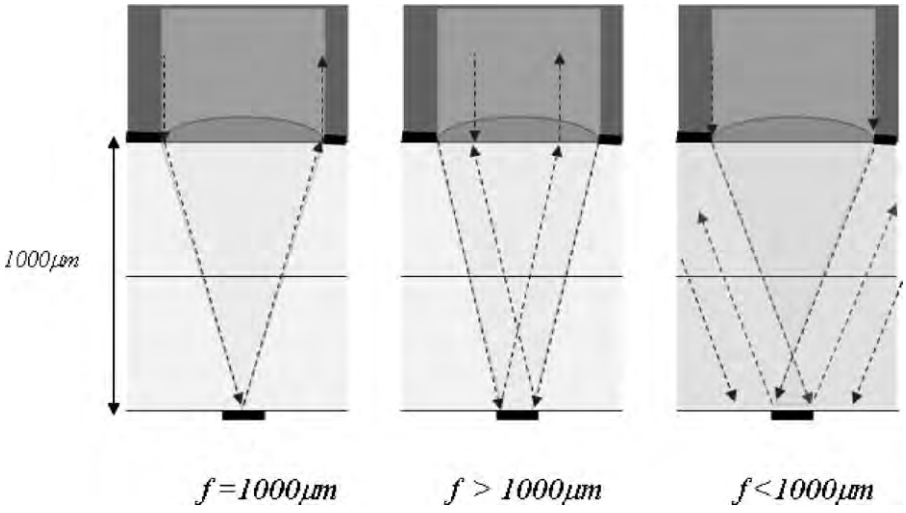

Fig. 3. Effect of focal length on the full reflection through aperture.

resulting pattern was transferred into the Cr/Au layer by wet etching. The channels on the glass wafer were wet-etched with 49% HF solution, followed by removal of the chrome and gold film sequentially. The inlet holes for the reagents were drilled by sand blasting followed by thermal bonding of lid glass wafer to create fluid channel. After chrome layer was deposited by electron beam evaporator on both sides, aperture for microlens and micro mirror were patterned and etched by chrome etchant. Microlens was fabricated by re-flowing method with thick AZ9260 photoresist (Clariant Corp.). All fabrication steps are illustrated in Fig. 5. Photoresist was spin-coated followed by two-step soft baking at 85 and 115 °C. After microlens pattern was developed, the 15 μm high cylinder-shaped photoresist was re-flowed in oven at 120 °C to be 21 μm high microlens. Fig. 6 shows fabricated microfluidic chip with microlens and mirror at both sides. Each chip has 5 sets of mirror and microlens. The photograph through microlens shows the magnified channel, which confirms that lens was properly fabricated.

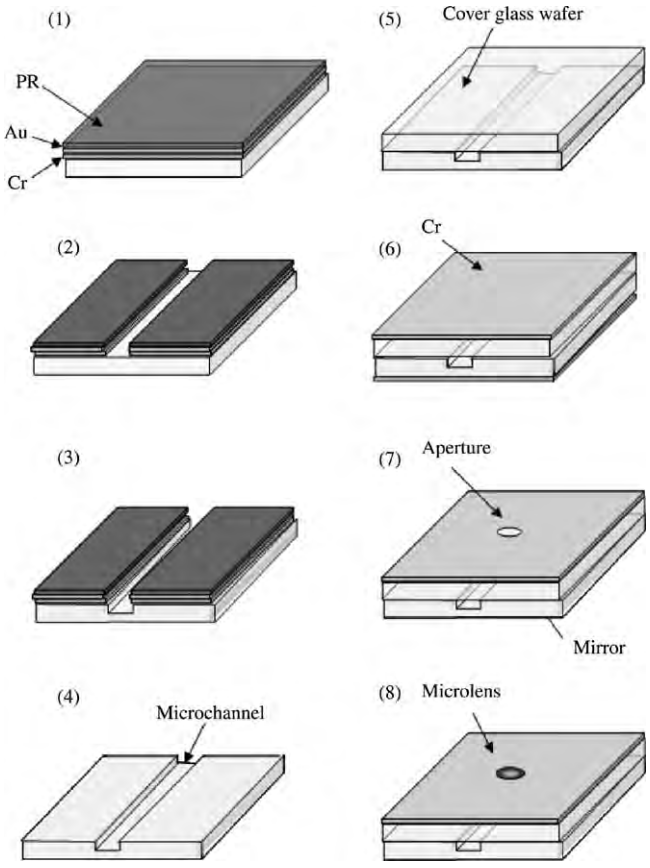

Fig. 5. Fabrication process of microlens and mirror on glass chip.

2.3. Experimental set-up

Conceptual drawing in Fig. 7 illustrates experimental set-up to obtain the electrical signal of fluorescence intensity. Fluorescence signal from microfluidic channel was collected by PMT (photomultiplier tube, H5784, Hamamatsu Corp.) through 10× objective lens of fluorescence microscope with dichroic mirror and bandpass filter. The field of view of objective lens was about 400 μm by 350 μm, and 10 mW green (532 nm) pumped laser diode (Worldstar Tech.) was used which was divided by beam splitter (50:50). The beam through splitter is used to monitor the laser power and the reflected beam excited the fluorescence dye. Fluorescence dye was TMR (tetramethyl rhodamine), which is excited at 550 nm and emitted at 590 nm. Emitted fluorescence was collected through bandpass filter (Fluorescence mirror unit U-MWIY2, Olympus), whose transmittance is less than 0.05% at 532 nm and more than 90% above 610 nm. The chips were cleaned with NaOH, HCl, deionized water and running buffer for 10 min, respectively before experiment. The chip was placed in the holder, mounted on the microscope stage. To sip diluted fluorescence dye, vacuum was applied on the waste reservoir and the area of microlens was then focused by microscope. Fluorescence digital CCD camera (Hamamatsu Photonics KK) was used to obtain the image of fluorescence and PMT signal data was gathered by A/D converter and processed in PC.

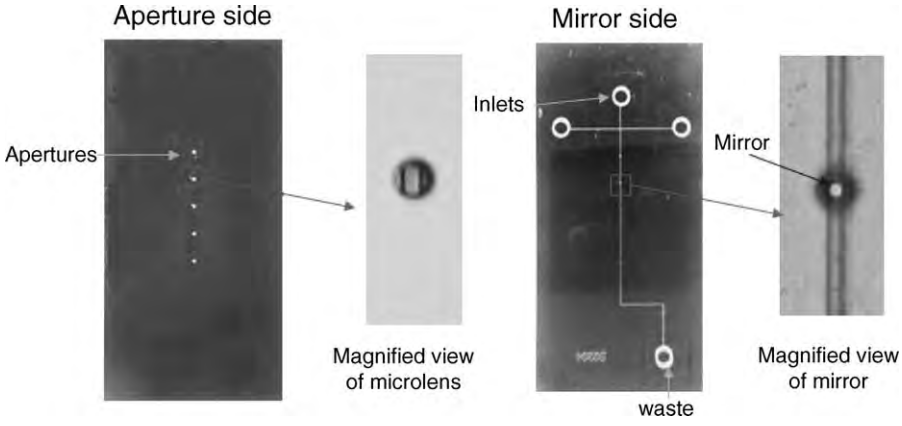

Fig. 6. Fabricated microfluidic chip with microlens and micro mirror: The microfluidic channel was designed for CE of DNA. The channel is magnified by microlens. Mirror is shown at magnified view of mirror as a bright circle on the channel.

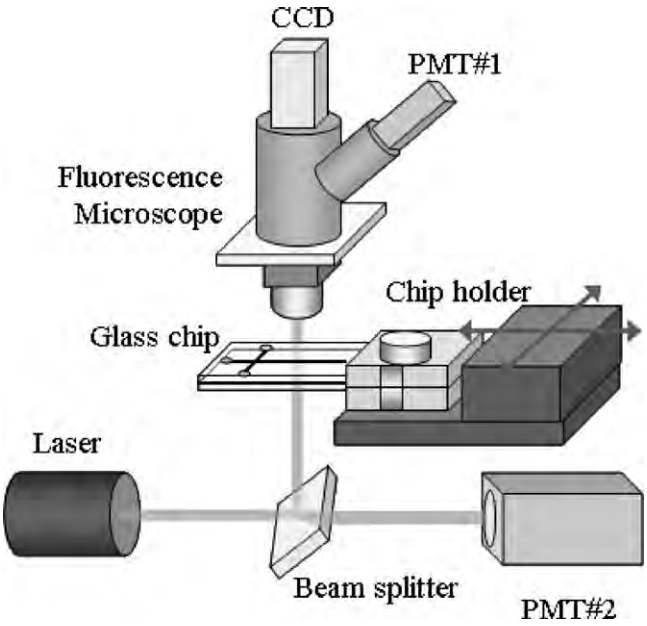

Fig. 7. Concept of experimental set-up: laser of 488 nm wavelength excited microchannel with microlens. PMT #1 and #2 monitored exciting beam power and fluorescence.

3. Results and discussion

3.1. Focus of microlens and scattering of channel

After fabrication, the focused shape was observed to verify the microlens focusing and the feasibility of the suggested optical layout. The focal length of microlens without microchannel was measured as 1.3 mm by moving the focus of microscope. Focused shape by microlens was perfect circle as shown in Fig. 8(a). However, when the microlens focused the beam with D-shaped microfluidic channel caused by isotropic etching, a parasitic elliptical beam was observed due to the refraction of the quarter circle at the side of channel. The shapes of focused beam with channel filled with phosphate buffer saline (PBS) solution and air are shown in Fig. 8 (b) and (c), respectively. Since it was expected that micro mirror cannot block the incident beam perfectly, we analyzed the intensity of the ellipse. Although the graph was not shown here, fortunately it was less than one third of that of focused circle, which means the mirror can reflect more than half of incident beam and the suggested scheme is still effective.

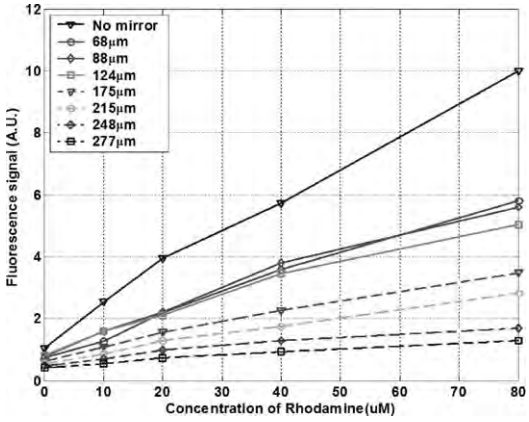

Fig. 9. Fluorescence intensity with respect to the concentration of rhodamine: the diameter of mirror was changed from 68 to 277 μm.

3.2. Fluorescence signal and background

Fluorescence intensity of microchannel was measured by PMT with the variation of fluorescence dye concentration. The concentration of fluorescence dye was changed as 0, 10, 20, 40, and 80 μM. The reason for relatively high concentration of fluorescence dye was due to its tiny volume. The volume of the channel through the microscope with 10× magnification lens was hundreds of picoliter of sample. The limit of microfluidic fluorescence detection in our system is in the range of 1–10 nM depending the quantum efficiency of dye, quenching of sample and bleaching of dye. Additionally the absorbance of microlens decreased the exciting optical intensity and the fluorescence signal was weaker than direct exciting. Two chips were used to investigate the effect of mirror diameter and 10 cases of mirror size were tested. Fig. 9 shows that the intensity signal increases with the concentration and decreases with the diameter of mirror. However, the plotted lines were overlapped in the range of 68–124 μm, where the mirror was smaller than the long axis of ellipse. Since both filtered incident beam and fluorescence were detected at photo detector, the background noise of elliptically focused beam was added to fluorescence signal. When the mirror was larger than the ellipse, the intensity became smaller since it prevented the fluorescence from reaching the photo detector.

The limit of detection of fluorescence measurement is determined from the detection floor, which is defined as three times the total noise σ_N above the background signal when the microchannels were filled with buffer solution [9]. Since

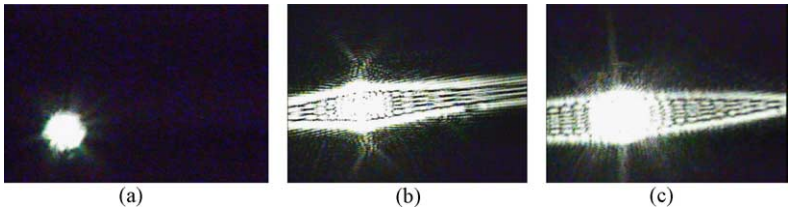

Fig. 8. Photographs of focused beam without channel (a), with channel filled with PBS (b), and with channel filled with air (c).

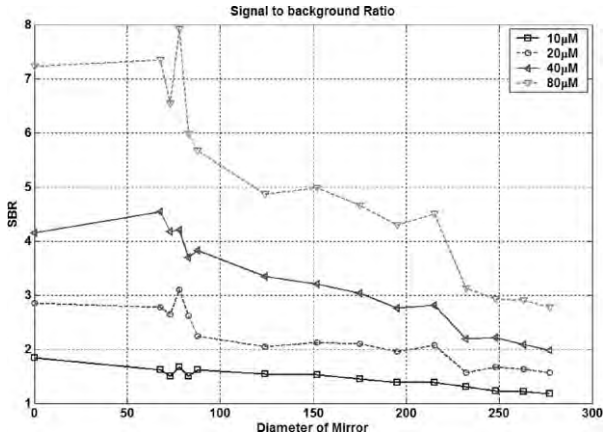

Fig. 10. Ratio of signal to background with respect to the diameter of mirror: the concentration changed as 10, 20, 40, and 80 μM.

the total noise is usually proportional to background signal, optimal mirror diameter for the highest signal to noise ratio can be found where signal to background ratio is maximum. After calculating background with linear fitting, signal to background ratio is plotted in Fig. 10. Maximum ratio was attained around 78 μm diameter which was that of focused center beam in Fig. 8. That means the optimal diameter of mirror is same as the focused beam. However, the signal to background ratio at optimal diameter was not far beyond the mirror-less microlens chip due to the autofluorescence of photoresist microlens and the refraction of microchannel as mentioned before. Fig. 11 shows the fluorescence images at the bottom for the case of buffer (a) and 10 μM fluorescence dye (b). Maximum intensity values of the buffer and rhodamine were about 40 and 60, respectively, which shows the fluorescence of lens is quite large background noise. Even the elliptical shape from exciting beam is not clearly seen due to the lens background fluorescence. The fluorescence from lens can be diminished by using long wavelength fluorescence dye like Cy5 (emission at 645 nm) since photoresist absorbs smaller light at long wavelength as shown in Fig. 12. Another solution is changing the material of lens, for example, glass or polymer with low fluorescence such as Polymethylmethacrylate (PMMA) or cyclo-olefin copolymer (COC). If excessive background noise from lens is removed, reflection of micro mirror will effectively enhance the signal to background ratio.

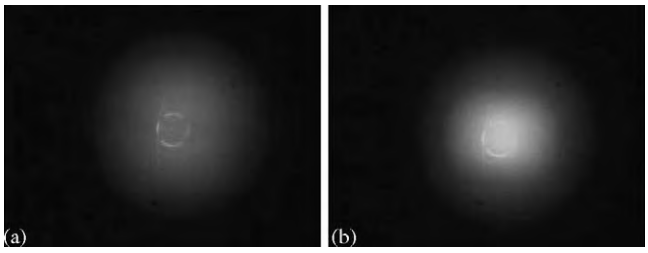

Fig. 11. Images and intensity graphs collected by fluorescence digital CCD camera for buffer (a) and 5 μM rhodamine (b).

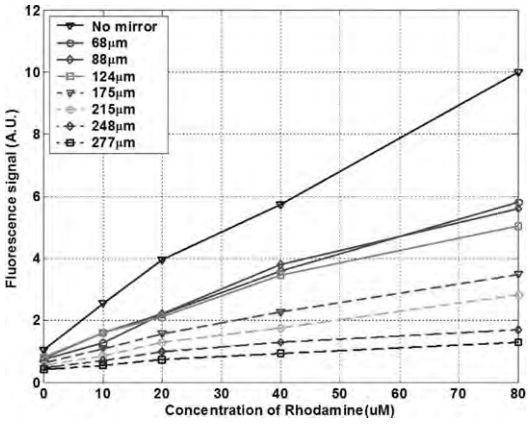

Fig. 12. Absorbance ratio of 15 μm thick photoresist (AZ9260) with respect to the wave length of incident beam (400–100 nm).

3.3. Analysis of light propagation

Through modeling of light intensity propagation, signal to background ratio was calculated theoretically without fluorescence of lens. Fig. 13 shows the propagation of light. Intensity of excitation light (I_E) was supplied by laser and some are absorbed by fluorescence dye and the other (I_T) is transmitted to bandpass filter. Filtered transmitted light then become background noise intensity (I_B). After absorbing, fluorescent dye emits fluorescence light (I_F), which sum of lost fluorescence (I_L), collected fluorescence is by filter (I_C), and blocked fluorescence by mirror (I_M). I_L denotes the portion that is not collected by sensor due to the geometry. Fluorescence signal (I_S) is determined by collected fluorescence I_C and transmittance of filter at fluorescence wavelength. Absorbance ratio,

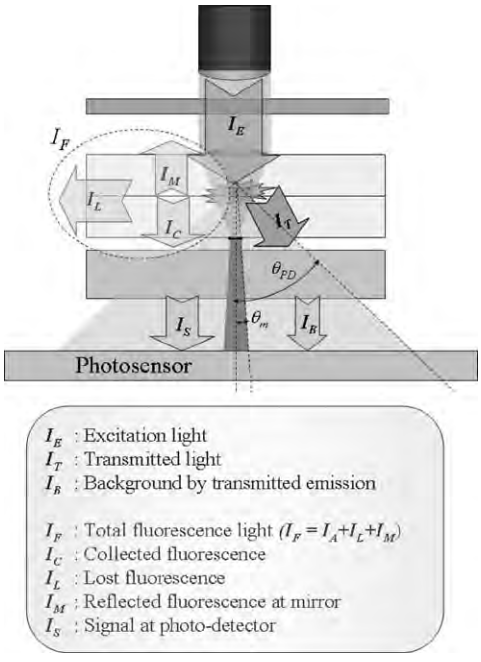

Fig. 13. Model of light intensity propagation.

Table 1
Simulation parameters

	Meaning	Value	Unit
ε	Extinction coefficient	107000	$cm^{-1}\,M^{-1}$
ϕ	Quantum efficiency	0.4	
c	Concentration	1	μM
t_{filter}	Thickness of filter	3	mm
τ_f	Filter transmission ratio at 570 nm	0.98	
τ_e	Filter transmission ratio at 543 nm	0.02	
d	Depth of channel	30	μm
t_{glass}	Thickness of glass wafer	0.5	mm
r_{PD}	Radius of photo sensor	5	mm
r_{focus}	Radius of focused beam	45	μm

A is defined by Lambert–Beer law [9].

$$A = \log \frac{I_E}{I_T} = \varepsilon l c, \tag{2}$$

where ε is the extinction coefficient, c the concentration, and l the depth of channel. Then intensity ratio of fluorescence and transmitted light is described as:

$$\frac{I_F}{I_T} = \phi(10^A - 1), \tag{3}$$

where ϕ is the quantum efficiency of fluorescence dye. Usually in microchannel, the ratio is around on the order of 10^{-5}, hence a bandpass filter with very low transmittance is required to separate the transmitted light from fluorescence. If transmitted light is removed by the suggested optical configuration, required specification of bandpass filter can be relieved. Considering the fluorescence loss and blocking transmitted light at mirror, the ratio of signal and background noise is derived:

$$\frac{I_S}{I_B} = \phi(10^A - 1)\, 10^{(OD(e)-OD(f))} \frac{\cos\theta_m - \cos\theta_{PD}}{2\tau_M}, \tag{4}$$

where θ_m and θ_{PD} are angles of mirror and photo sensor as shown in Fig. 13. OD(e) and OD(f) denote optical density of filter at the wavelength of exciting light and fluorescence, respectively. τ_M is virtual transmittance of mirror and defined as:

$$\tau_M = (1 - R_{SC})\tau_{mirror} + R_{SC}\tau_{SC}, \tag{5}$$

where R_{SC} is the ratio of scattering at mirror and τ_{SC} the transmittance ratio of scattered light. τ_{mirror} denotes the ratio of transmitted light at mirror and defined as:

$$\begin{aligned} \tau_{mirror} &= 1 - \frac{r_m^2}{r_{focus}^2}, \quad r_m < r_{focus} \\ \tau_{mirror} &= 0, \qquad\qquad \text{otherwise} \end{aligned} \tag{6}$$

where r_m is the radius of mirror and r_{focus} the radius of focused beam. Simulation parameters and values are defined in Table 1 and the calculation results are shown in Fig. 14. It shows that tremendous improvement in signal to background ratio is possible theoretically if it has no channel refraction and lens fluorescence background.

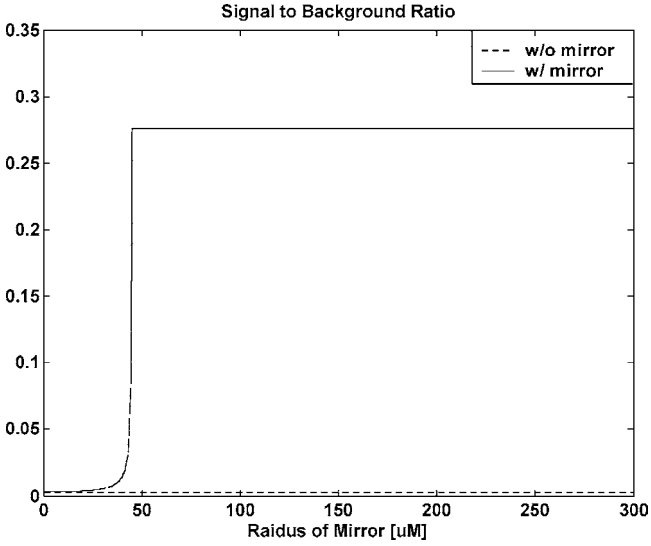

Fig. 14. Theoretical improvement of signal to background ratio with microlens and mirror.

4. Conclusion

A microfluidic chip with microlens and mirror was designed, fabricated and characterized to remove background noise from exciting light. It has advantages of low cost fabrication compared with custom-designed photo sensor or inclined beam set-up since it uses commercial photosensor and no optical filter deposition. Both experiment and simulation shows that the optimal diameter of mirror is almost same as that of focused beam. However, dramatic improvement in signal to background noise ratio was not observed in experiment due to the fluorescence of lens and refraction of channel. The problem can be solved by the lens with low autofluorescence and microfluidic channel with vertical side-wall using soft lithography or injection molded biochip.

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Biographies

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Eun Gyung Yang received her Master's degree in bioorganic chemistry from Seoul National University. She proceeded to Stanford University, where she obtained her PhD in biophysical chemistry in 1994. After a post-doctoral fellowship at Stanford Medical School, she joined SRI International where she worked on peripheral neuropathy and biosensor development. She then spent 2 years working in the multi-disciplinary team for HTS assay development based on microfluidic technology at Caliper Technologies Corp. She is now a senior research scientist in Life Sciences Division at KIST in Korea. Her current research interests include development of miniaturized biochemical assay systems and biochip-based functional proteomics.

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Ji Yoon Kang obtained his PhD in mechanical engineering from Seoul National University, Korea, in 1997. After working as a member of research staff at Samsung Advanced Institute of Science and Technology, he has been a senior research scientist at KIST since 2001. He was a post-doctoral associate studying plastic biochip at University of Cincinnati from 2002 to 2003. He is currently interested in microfluidic biochemical assay system, nano-biosensors, and cell based assay lab-on-a-chip.