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Journal of Chromatography A, 1106 (2006) 89–93
JOURNAL OF CHROMATOGRAPHY A
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Miniaturized thermal lens and fluorescence detection system for microchemical chips

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Available online 8 November 2005

Abstract

We have developed a miniaturized two-way detection system using thermal lens and fluorescence spectroscopies for microchip chemistry. The system was composed of laser diode (LD) modules, fiber-based optics combined with a gradient index lens, and miniaturized detection units for thermal lens and fluorescence signals. The detection limits in the thermal lens and fluorescence spectroscopies were 6.3×10^{-9} M for Ni(II) phthalocyanine tetrasulfonic acid and 3.0×10^{-9} M for cy5, respectively. The performance of the system with the miniaturized thermal lens was equivalent to that of a conventional thermal lens microscope. The fluorescence sensitivity was comparable to sensitivities offered by conventional miniaturized systems.

Keywords: Thermal lens detection; Fluorescence detection; Microchip; Microchannel

1. Introduction

Recent years have seen great interest in microchemical systems, and integration of chemical processes including mixing, chemical reaction, and separation on a small glass or polymer microchip has been rapidly developed [1,2]. Highly sensitive detectors are indispensable for such research, and because conventional detection systems are much larger compared with the size of the microchip, miniaturization of the detection systems is required for portable microchip analysis systems.

Optical detection methods are commonly used in microfluidic devices because they are simple and reliable. Spectrophotometric detection of absorbance has wide applicability, but its sensitivity is insufficient due to the short optical path length in the microchemical chips. Therefore, fluorescence and thermal lens methods have been selected as sensitive detection methods in microchips.

In the fluorescence method, radiative relaxation processes of target molecules are measured while non-radiative relaxation processes are detected in the thermal lens method. The fluorescence method provides information on both absorption and fluorescence spectra and, therefore, is selective. However, because quantum yield of non-radiative processes can be regarded as unity for most chemical species except for fluorescence dyes and fluorescence labeling reagents, the thermal lens method is widely applicable.

In terms of miniaturization of the fluorescence detection systems, various designs with optical fibers and microavalanche photodiodes integrated onto a microchip have been proposed [3–13]. For example, Hubner et al. [5] integrated an optical fiber into the microchannel and fabricated optical waveguides, and the detection limit of fluorescein was reported as 250 pM and of BODIPY 650/665X as 100 nM. Liang et al. [6] measured 3 nM of fluorescein by connecting fibers to longitudinal paths of the channels, and Chabinyc et al. [7] detected 25 nM of fluorescein by encapsulating an optical fiber and a microavalanche photodiode in poly(dimethylsiloxane) (PDMS) substrate in proximity to the channel. Optical microlenses of various materials were

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doi:10.1016/j.chroma.2005.09.091

installed on top or inside the microchips. These microlenses coupled with optical fibers were used to focus the light precisely into the channel on a portable chip. Detection limits of 0.1 g l^{-1} of FITC-labeled albumin by Camou et al. [8] and a few nM of cy5 by Roulet et al. [9–11] have been described.

Clearly, studies on miniaturization of fluorescence detection systems are available, but applicable compounds are limited if we only depend on fluorescence detection. Recently, we have reported miniaturized thermal lens systems with fiber optics and small gradient index lenses (Selfoc microlenses) [14–16]. The Selfoc microlenses were arranged closely onto the top plate of the microchemical chip. If a sensitive portable analyzer which provides complementary information from thermal lens and fluorescence spectroscopies is realized, it should greatly contribute to the progress in chemistry in microchips. By utilizing the two-way sensitive detection method, various spectrophotometric and fluorescent labeling reagents can be applied.

In this study, we have developed a small detection system for the two complementary methods, thermal lens and fluorescence spectroscopies. The optical system based on optical fibers and Selfoc microlenses was used for both methods. The excitation beam was introduced into the microchannel using a Selfoc microlens from the top plate, and a thermal lens detector was set coaxially under the microchip. The fluorescence collection geometry was also studied, and a two-way detection system which has a fluorescence detection path perpendicular to the optical axis of the excitation beam was realized. The lock-in detection system was used in thermal lens and fluorescence spectroscopy to improve the sensitivity. We have evaluated the detection limits and determination limits by using Ni(II) phthalocyanine tetrasulfonic acid, tetrasodium salt (NiP) and a standard fluorescence labeling dye cy5 for thermal lens and fluorescence systems, respectively.

2. Experimental

In the thermal lens spectroscopy, an aqueous solution of Ni(II) phthalocyaninetetrasulfonic acid, tetrasodium salt (NiP) (ε_{659} = 43,700) (Aldrich, Milwaukee, WI, USA), was used. In the fluorescence spectroscopy, an aqueous solution of cy5-monofunctional reactive dye (Amersham Bioscience, Piscataway, NJ, USA) was used. The dye cy5 has a maximum absorption wavelength of 649 nm (ε_{649} = 250,000), maximum emission wavelength of 670 nm, and quantum yield of 0.28. The solutions were introduced into a microchannel (width, 150 μm; depth, 200 μm) by using a microsyringe pump.

2.1. Design and principle

Fig. 1a shows the radial refractive index distribution $N(r)$ of the Selfoc microlens. There was a gradient refractive index within the lens, and the index on the center axis is shown as n_0. Fig. 1b illustrates a design of the miniaturized thermal lens and fluorescence analysis system on a microchip. Two laser diodes (LDs) were installed in the system. The excitation and probe beams had wavelengths of 658 nm and 785 nm, respectively. The probe beam was only used in the thermal lens spectroscopy.

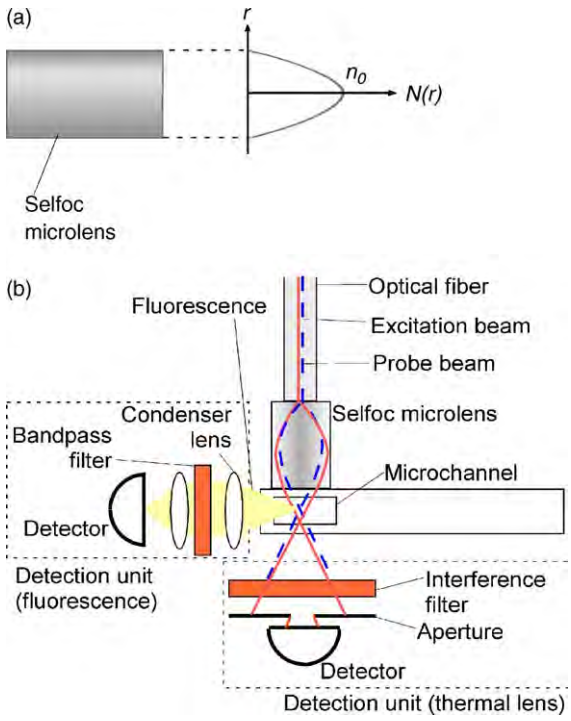

Fig. 1. (a) Radial refractive index distribution $N(r)$ of Selfoc microlens. (b) Design of miniaturized thermal lens and fluorescence analysis system.

The intensity of the excitation beam was modulated by voltage switching of the LD driver. The excitation and probe beams were coaxially aligned and introduced into the Selfoc microlens.

In the thermal lens spectroscopy, the detector was arranged coaxially to the optical axis. When a part of the excitation beam was absorbed by non-fluorescent sample solution, the absorbed light energy was released as thermal energy and a temperature gradient was produced around the excitation beam axis. The temperature gradient was equivalent to the gradient of the refractive index because refractive index change is proportional to temperature change. The gradient of the refractive index acted as a transient optical lens, which is called the thermal lens effect. The degree of the thermal lens effect was proportional to absorbance in the detection volume and could be measured by a locus change of the probe beam. The locus change was detected by measuring the light intensity after passing through an interference filter and an aperture. Here, the focal point of the probe beam was set slightly below that of the excitation beam in order to optimize signal sensitivity.

In the fluorescence spectroscopy, the same excitation module as in the thermal lens spectroscopy was used. The fluorescence detection path was set perpendicular to the optical axis. A small-sized condenser lens was used to collect the fluorescence, and a band-pass filter was used to cut the scattered excitation light.

2.2. Miniaturized optical system

Fig. 2a and b are a schematic diagram and a photograph of the miniaturized thermal lens and fluorescence detection system. The system was composed of two laser diodes (excitation: HL6501MG, 35 mW; probe: 7859MG, 35 mW) (Hitachi, Tokyo,

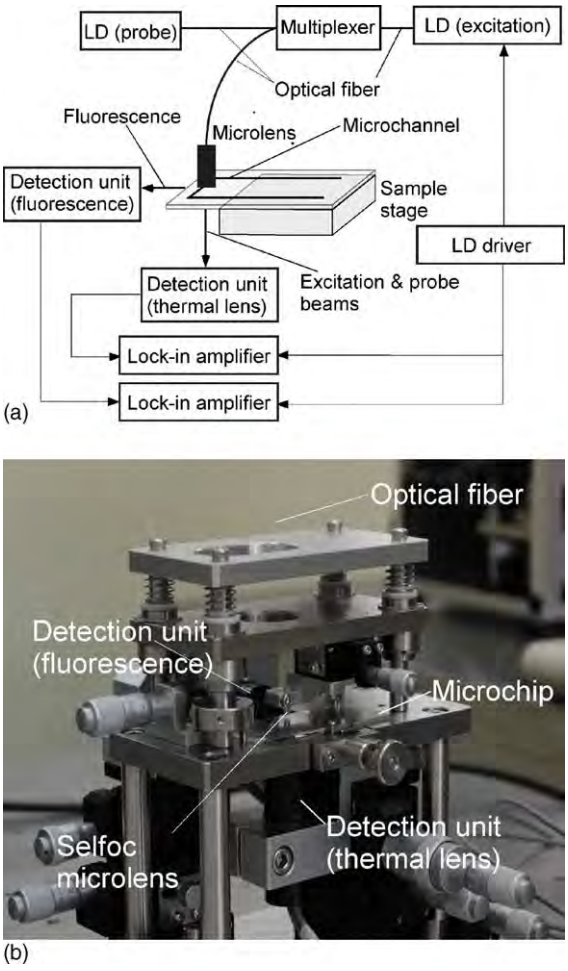

Fig. 2. (a) Schematic diagram of the miniaturized thermal lens and fluorescence analysis system. (b) Photograph around the sample stage.

Japan), wavelength division multiplexing (WDM)-type optical multiplexer (Nippon Sheet Glass, Osaka, Japan), an optical fiber (FiberCore, Southampton, UK, outer diameter: 125 μm, core diameter: 4 μm), Selfoc microlenses(diameter, 1 mm; height, 2.33 mm; NA, 0.27; Nippon Sheet Glass), a sample stage for a microchemical chip, and detection units for thermal lens and fluorescence spectroscopies. The thermal lens and fluorescence signals were measured using lock-in amplifiers.

The detection unit for the thermal lens spectroscopy was set in the optical path of the probe beam. In order to detect the probe intensity, a silicon photodiode (S1337-33BR, Hamamatsu Photonics, Hamamatsu, Japan) was set after passing though an interference filter (780FS20-12.5, Andover) and a pinhole of 1 mm in diameter in front of the detector. The interference filter blocked the excitation light and transmitted the probe beam. The pinhole was set in the center part of the active area of the photodiode. We have optimized the pinhole size to obtain sufficient photon flux and accuracy of the measurement. The electronic signal from the photodiode was fed into a current-to-voltage (*I–V*) amplifier and synchronously amplified with a lock-in amplifier (LI-575, NF, Yokohama, Japan).

The detection unit for fluorescence detection was arranged perpendicular to the optical axis and positioned close to the edge of the microchip. It was composed of two aspheric glass condenser lenses (Sigma Koki, Tokyo, Japan), a band-pass filter (682DF22, Omega Optical, Brattleboro, VT, USA), and an avalanche photodiode (APD) (S3884, Hamamatsu Photonics). These components were fixed by sectional holders. The working distance of the condenser lens was 10.5 mm and numerical aperture of fluorescence collection was 0.43. The sensitivity of the APD was 50 A/W and active area of the APD window was 1.77 mm^2. The fluorescence signal from the APD was amplified by an APD amplifier (Neoark, Tokyo, Japan) and fed into a lock-in amplifier.

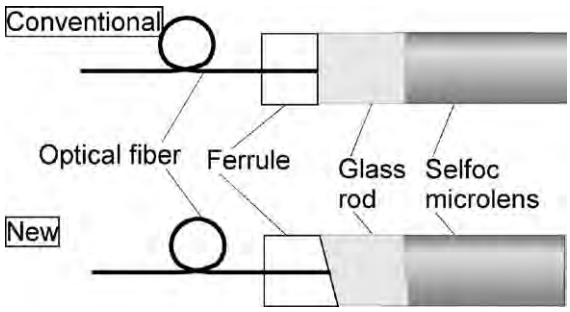

Fig. 3. Comparison of connection method of the optical fiber and Selfoc microlens.

2.3. Connection

The objective Selfoc microlens was coaxially connected with the optical fiber by using a capillary tube and ferrule, and then supported by a lens holder. The characteristics of the Selfoc microlens can be controlled arbitrarily by changing ion species, spatial distribution of implanted ions and lens height. The radial refractive index gradient of the Selfoc microlens was designed to obtain adequate chromatic aberration, which is required to shift the focal points of the excitation and probe beams in the thermal lens spectroscopy. The difference between the focal points was set to 37 μm.

For easy connection between the optical fiber and the microlens, both end-faces of the cylindrical Selfoc micro lens were set perpendicular to the optical axis. In this optical configuration, however, the excitation and probe beams were reflected at the end-face of the microlens although an anti-reflection coating was used. The reflected beams went backward to the LDs and stability of the LD emission deteriorated. Since the background noise due to the instability dominates a limit-of-detection, the instability due to the reflected light should be lessened.

In order to avoid reflection, a new connection method between the optical fiber and Selfoc microlens was designed. Fig. 3a shows the conventional method, where the fiber and the microlens were connected through a glass rod having a cutting plane perpendicular to the optical axis. Fig. 3b illustrates the new method, where the glass rod had an inclined cutting plane to the optical axis. While the reflection light can be easily introduced to the LD for the perpendicular cutting plane, the reflection light refracted at the inclined cutting plane and, therefore, little part of the reflection light was introduced to the LD. By utilizing the

new method, the reflection light intensity was reduced from 3% to 0.001%.

2.4. Sample stage

In order to arrange relative positions between the microchip and the microlens and between the microchip and the detection units, the sample stage (Fig. 2b) should be composed of three parts for positioning the microlens, the microchip and the detection units. The bottom part of the stage held the thermal lens and fluorescence detection units. Each detection unit had micrometers to adjust relative position. The middle part of the stage fixed the microchip and its holder. The upper part of the stage fixed the Selfoc microlens.

2.5. Size of the miniaturized system

The sample stage was 110 mm × 170 mm × 178 mm. The LD module and *I–V* amplifier had overall dimensions of 100 mm × 140 mm × 40 mm. Excluding the lock-in amplifier and the APD amplifier, the total system in its carrying case and the syringe pump had dimensions of 230 mm × 260 mm × 300 mm. In order to realize further miniaturization down to an on-the-palm-sized system, sizes of the electric power supply unit, the LD driver, the APD amplifier, the *I–V* amplifier, the lock-in amplifier, the multiplexer and the syringe pump must all be cut.

3. Results and discussion

Fig. 4a shows the dependence of the thermal lens signal intensity on the concentration of NiP. The power of the excitation and the probe beams just below the Selfoc microlens were set as 3.4 mW and 0.22 mW, respectively. The time constant of the lock-in amplifier was set as 4.0 s. The detection limit (S/N = 2) and determination limit (2σ) were estimated to be 6.3×10^{-9} M and 6.5×10^{-8} M, respectively. In our previous report using the flat end-face connection of the microlens, the detection limit and determination limit were 7.7×10^{-9} M and 1.1×10^{-7} M for NiP [16]. By utilizing the inclined connection to avoid reflection, the detection and determination limits were slightly improved. Although the detection limit of the present system was slightly inferior to that reported for thermal lens microscopy [16,17], the system had sufficient performance despite its miniaturization.

In the fluorescence spectroscopy, we set the detection unit perpendicular to the excitation beam axis (90° configuration). We also investigated fluorescence detection by using a detector set in the excitation beam axis (0° configuration), but the background signal was 10 times higher than that in the 90° configuration. The high background signal in the 0° configuration deteriorated the detection limit.

Fig. 4b shows a calibration curve in the 90° configuration for cy5 aqueous solution. The power of the excitation beam after passing through the Selfoc microlens was set as 0.60 mW. The time constant of the lock-in amplifier was set as 1.25 s. While an avalanche photodiode was used in the fluorescence detection, a positive-indulator-negative (PIN) photodiode was used in the thermal lens detection to measure relatively high background signal. The difference might affect difference in the error bars in Fig. 4a and b. The detection limit (S/N = 2) and the determination limit (2σ) were estimated to be 3.0×10^{-9} M and 3.8×10^{-9} M, respectively.

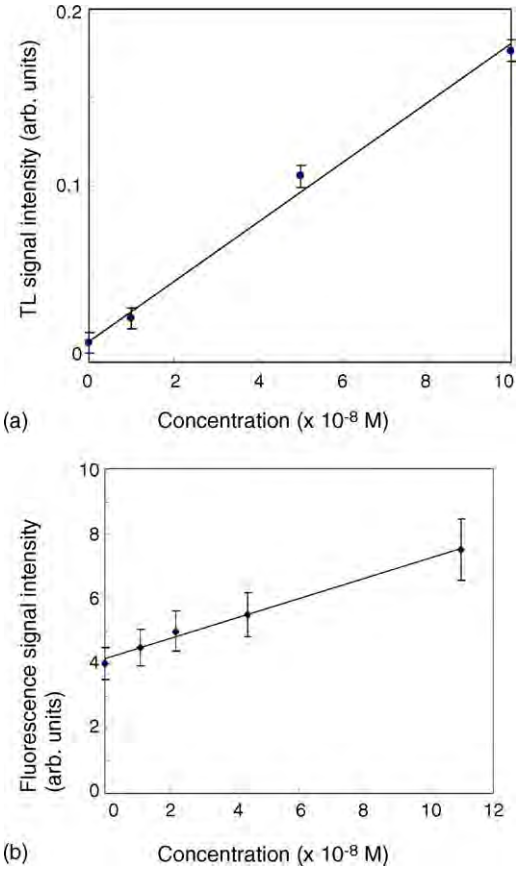

Fig. 4. (a) Thermal lens signal dependence on NiP concentration. (b) Fluorescence signal dependence on cy5 concentration.

Using a conventional confocal microscope and a photomultiplier tube, the detection limit for cy5 in a microchannel was reported as 9 pM [18]; it was 20 pM using a CCD camera and a flame hydrolysis deposition chip of glass on silica [19]. As for miniaturization of the detection system, Roulet et al. [11] had a detection limit for cy5 of 3.3 nM using a microlens array fabricated into a microchip, with a He–Ne laser as the light source and a PMT for the detector. In our fluorescence detection system, we have achieved miniaturization by reducing the size of the excitation source and detection units without resorting to micro-optics fabricated into the microchip and photomultipliers. Although the detection limit of the present system was inferior to that of the confocal laser microscope, the system showed good performance as an integrated fluorescence detector for microchip chemistry.

4. Conclusions

We have developed the miniaturized optical detection system for microchip chemistry which can detect both thermal lens and fluorescence signals. The present detection system

was constructed based on fiber optics and Selfoc microlens technology. The detection limits of 6.3 nM for the thermal lens signal (NiP) and 3.0 nM for fluorescence signal (cy5) were achieved by utilizing laser diodes and photodiodes. The thermal lens and fluorescence signals are generated via non-radiative and radiative relaxations after light absorption and, therefore, these signals are complementary. Since the present system can detect both thermal lens and fluorescence signals, it is expected to be applicable for various kinds of target molecules.

Acknowledgements

We would like to acknowledge Mr. Yoshikuni Kikutani of Kanagawa Academy of Science and Technology for help in microchip fabrication. The present work was partially supported by a grant from the New Energy and Industrial Technology Development Organization (NEDO) of the Ministry of Economy, Trade and Industry, Japan.

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