DEVELOPMENT OF A REFLECTED LIGHT
FLUORESCENCE UNIT FOR THE MICROFLUIDIC
DETECTION SYSTEM
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Abstract
We have developed a reflected light fluorescence unit for a microchip detection system. This fluorescence detection unit can be attached to a commercial fluorescence photometer. The unit's equipment includes optical fibers (Ø 0.8 mm), a stage that moves 10 mm in the X, Y and Z directions and a special lid for inserting capirally. The excitation light is irradiated on the bottom of the microchip from a close position through the fibers, and the emission is condensed with a lens and then detected by a photomultiplier tube. We have also examined the fluorometric determination of sulfite with N-(9-acridinyl)maleimide using this unit and a PDMS microchip. The detection limit of sulfite was 500 µM at the microchip. In a comparison of the absolute quantity in the detection volume, the microchip using our unit was better than the conventional cuvette (2.3 nmol and 156 nmol, respectively). It is considered that the fluorescence unit is an effective tool for biological analysis.

Keywords: Reflected light fluorescence unit, optical fiber, PDMS, NAM, sulfite

1. Introduction
Recently, micro and nano technologies have been widely applied in various scientific fields. Microchip technology for micro total analysis systems (µTAS)[1,2] has developed into one of the most exciting fields in analytical chemistry. Microchips based on microfluidic systems provide for the rapid analysis and operation of biological samples. Previously, we reported on the integration of a histamine release system on a poly(dimethylsiloxane)(PDMS) microchip retaining animal cells [3], but the detection of histamines has technical problems; i.e., it is difficult to gain sufficient emission for detection by conventional equipment because the sample volume irradiated by excitation light is very small. Therefore, we tried to develop a reflected-light fluorescence unit for a microchip system as a unique and highly sensitive detection method.
2. Apparatus

This unit was developed in collaboration with JASCO Corp. (JAPAN). The unit is attached to a commercial fluorescence photometer (FP-6500, JASCO Corp., JAPAN), and it is replaceable with the original cuvette holder. Schematic images of the unit are shown in Fig. 1a,b. Fluorescence was detected with the unit as follows. Firstly, microchip (A) was set on the stage (B) of the unit, which can be moved 10 mm in the X, Y and Z directions (Fig. 1b). Next, the excitation light from the source (C) (Xe, 150 W) through the slit (D) was introduced into optical fibers (.0.8 mm). Then the excitation light was irradiated on the bottom of the microchip from a close position (Fig. 1b). The fluorescence was condensed with the lens (H) installed under the movable stage and then was detected by the photomultiplier tube (PMT) (I). This unit is also equipped with a special lid for inserting capirally.

3. Experimental

Chemicals and Materials. To demonstrate the utility of the unit, we examined the fluorometric determination of sulfite with N-(9-acridinyl)maleimide (NAM) (shown in Fig 2). NAM reacts highly sensitively with sulfite and gives strong fluorescent derivatives. NAM was applied to determine sulfite in wine [4]. NAM was obtained from TCI (Tokyo, Japan), and NaHSO₃ was obtained from Kokusan Chemical Works, Ltd. (Tokyo, Japan).
Microchip Fabrication. Micro channels were fabricated originally using PDMS with a homemade glass template (76 mm x 26 mm). A schematic image of the micro channel (300 μm wide and 100 μm deep) is shown in Figure 3. The volume of the detecting chamber is 300 nL, and the width is wider than that of the channel (1.5 mm).

Sulfite Measurements. NAM and reaction buffer were prepared according to Akasaka’s method [4]. A standard solution of sulfite was prepared with NaHSO₃ in a borate buffer solution. For the calibration and determination of sulfite in the sample solutions, 1.2 mM NAM-acetone solution was added to a mixture of the sample solution and reaction buffer (pH 8.8). A syringe pump was used to fill the micro channel with the borate buffer solution containing sulfite and NAM, and excited the solution at 360 nm and detected the emission at 432 nm. The optimum point of the fluorescence measurement was set by using the X, Y and Z stage.

4. Results and discussion

The flow rates of the solutions were set at 1 μl/min, and then the fluorescence of NAM-sulfite was measured by using the novel unit at the optimum position. The calibration curves of sulfite were compared with the microchip and the conventional micro cuvette (3 x 3 x34 mm, sample volume: 200 μL) (Fig. 4(a), (b)). The curves of NAM-sulfite using this unit and the conventional cuvette exhibited good linearity. The minimum concentrations detected were 50 μM at the conventional cuvette and 500 μM at the microchip. In a comparison of the absolute quantity in the detection volume, the microchip using our unit was better than the conventional cuvette (2.3 nmol and 156 nmol, respectively). This unit can choose the excitation and emission wavelength arbitrarily without the optical filter generally used in reflected light fluorescence equipment. Although the unit is a trial product, high sensitivity on an absolute quantity was observed. It is considered that the fluorescence unit is effective for biological analysis.
5. Conclusions

We have developed a reflected light fluorescence unit for a microchip detection system. Characteristics of this unit are 1) high energy irradiation by the optical fiber at a close position, 2) without optical filters and 3) condensation of the emission using a lens. To demonstrate the utility of the unit, we have examined the fluorometric determination of sulfite with NAM. The calibration curves of NAM-sulfite using this unit and a conventional cuvette exhibited good linearity. The detection limits of sulfite were 50 μM at the conventional cuvette and 500 pM at the microchip. In a comparison of the absolute quantity in the detection volume, the microchip using our unit was better than the conventional cuvette (2.3 nmol and 156 nmol, respectively). Although the unit is a trial product, high sensitivity on an absolute quantity was observed. It is considered that the fluorescence unit is effective for biological analysis, e.g., fluorometric detection of histamines.

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