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Sub-Zeptomole Detection in a Microfabricated Glass Channel by Thermal-Lens Microscopy

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A thermal-lens microscope which we developed was applied to an ultramicro quantity determination of a dye in an aqueous solution filling a microchannel (150 μm wide and 100 μm deep) fabricated in a quartz glass substrate. The detection volume, which corresponded to the confocal volume, was estimated to be 1.3 fl. The detection limit of the dye molecules was 160 ymol, and the calibration line showed good linearity in the sub-zmol-to-zmol region. This detection sensitivity is equivalent to that of laser-induced fluorometry. The thermal-lens signal measured in the microchannel was more stable than that measured in a liquid micro space between a slide glass and a cover glass, which was much wider than the microchannel. This may have been due to a suppression of convection in the microchannel. The thermal lens method can be applied to non-fluorescent chemical species, and is thus very suitable detection method for integrated chemistry systems.

Keywords Thermal-lens microscope, microchannel, integrated chemistry laboratory convection

Quantitative detection methods for ultramicro quantities of chemical species in a liquid micro space are highly desired in many fields, including analytical, biological and industrial chemistry. In particular, the development of such methods is indispensable to the monitoring of analytes in microfabricated fluidic devices (*e.g.* a microchip) and single cells *in vivo* or *in vitro*.

Microchips that carry out chemical and biochemical analysis procedures (miniaturized total chemical analysis systems, μ-TASs) have attracted much attention over the past few years. Microfabricated glass substrates are rapidly becoming a convenient platform with which to execute liquid-phase analyses and, consequently a variety of techniques based on electrophoresis have been implemented on microchips.[1,2] These days, open-channel electrochromatography[3,4], micellar electrokinetic chromatography[5-7], free-flow electrophoresis[8,9], capillary gel electrophoresis[10], enzyme and immunoassays[11,12], DNA sequencing[13-15], and PCR amplification[16,17] are being carried out on microchips. In these studies, the analytes are electrophoretically separated in the microchannels, and the separated chemical species are detected with high sensitivity by a laser-induced fluorescence method. Thus the function of these μ-TASs is as effective as possible, other micro liquid driving and chemical separation forces besides electroosmosis and electrophoresis are required, and highly sensitive and more widely applicable detection methods than laser-induced fluorescence are desired. The combination of electroosmotic fluid control, electrophoretic separation, and fluorometric detection methods can be adapted only to aqueous solutions, like biological substances or a few organic solvents.[18-20] Furthermore, an inhomogeneous spatial distribution of solutes may occur due to the electric field because of the difference in mobility. If various kinds of solutions including organic solvents can be transported while maintaining a homogeneous distribution of solutes, chemical reaction, separation, and ultrasensitive detection can be carried out in microchannels, and we can integrate many kinds of chemical laboratories beyond μ-TASs on a glass chip.

Among these elemental chemical operations, *i.e.*, liquid transport, mixing, reaction, separation, and detection, ultrasensitive detection is particularly important, because chemical integration, in whatever stage or complexity, is impossible without it. As mentioned above, desirable factors in the detection method are wide applicability, the possibility of *in vivo* or *in situ* use, and ultrahigh sensitivity in microchannels. Light-absorption spectrometry needs a long optical path length for highly sensitive detection, but microchannels do not have a sufficiently long optical path length;[21] moreover, there are problems with obtaining a strict optical alignment and design of the channels.[22] Although mass spectrometry has sufficient sensitivity, *in vivo* or *in situ* detections are impossible using this method, and it is essentially unsuitable for microchannel measurements. Although an electrochemical detec-

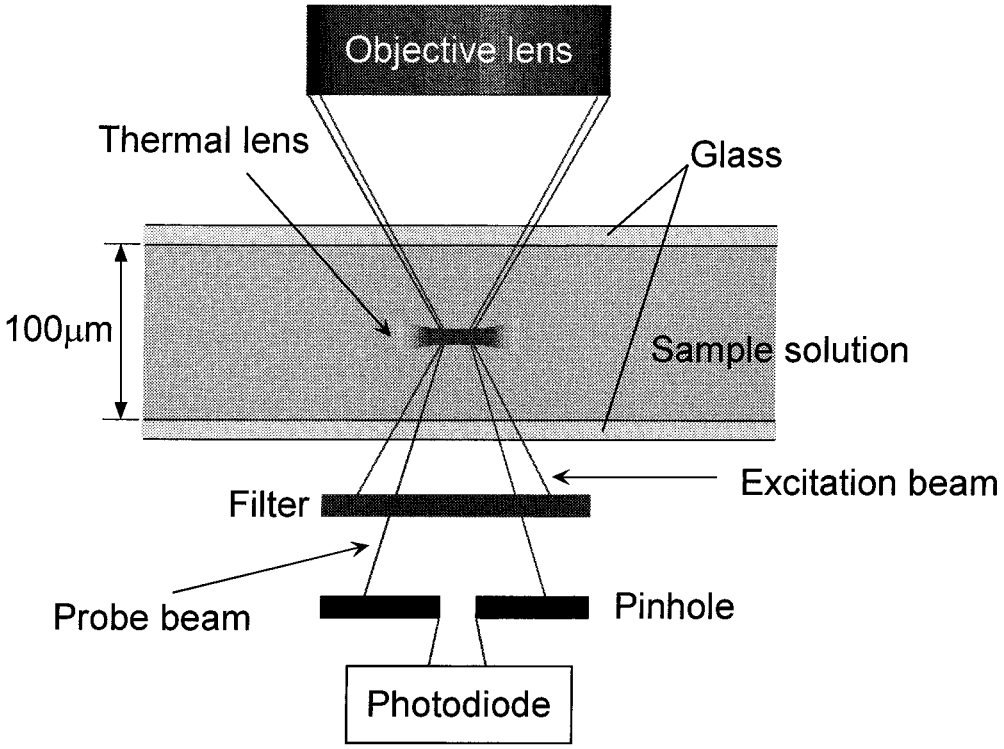

Fig. 1 Schematic illustration of the thermal-lens measurement in a microchannel.

tion method for micro quantities of a solution has been developed recently, obtaining ultrahigh sensitivity is difficult using this method. Laser-induced fluorometry, on the other hand, has ultrahigh sensitivity, but lacks wide applicability.

To overcome these problems of ultrahigh sensitivity and applicability, we have developed a photothermal microscope based on the thermal-lens effect, the thermal-lens microscope (TLM).[23-26] Photothermal spectroscopy offers highly sensitive methods to monitor the energy flow of heat resulting from the absorption of optical irradiation.[27] Because photothermal phenomena are caused by the most common relaxation process following absorption of light, the applicability of photoabsorption spectrometry is wide.

In this study, we applied TLM to ultrasensitive detection in a microchannel fabricated in a glass chip and investigated the determinations at sub-zmol-to-zmol levels. Furthermore, we found that the thermal-lens signal measured in the microchannel was more stable than that measured in much wider space, which showed that TLM is very suitable as a micro-space detection method in an integrated chemistry laboratory (ICL).

Experimental

A schematic illustration of the thermal-lens measurement in a microchannel is given in Fig. 1. The details of the optical configuration and the confirmation of the measurement principle were described elsewhere.[25,26] In brief, the coaxial beam of the excitation and the probe beam were focused into a liquid sample in the microchannel. The excitation beam formed a thermal-lens in the confocal region, and the probe beam, having a longer wavelength than the excitation beam, was focused just below the center of the thermal-lens. The ray locus of the probe beam moved in the convergent direction, as shown in the figure, because the thermal-lens normally acts as a concave lens, and the change in the optical power density passing through the pinhole could be measured.

A block diagram of the experimental set up is shown in Fig. 2. The excitation beam was the 488.0 nm emission line of an argon ion laser, which was mechanically chopped by a light chopper at 1.69 kHz. The probe beam was a He-Ne laser of 632.8 nm. These beams were made coaxial at the dichroic mirror, and were introduced into the optical microscope (×10, N.A. 0.80). An optical band-pass filter filtered the coaxial beams passing through the microscope, and only the probe beam was detected by a photodiode behind the pinhole. The glass chip was mounted on a 3-D stage, which could be controlled in 1 μm steps in each direction, and the step was precise enough for positioning the foci of the laser beams.

The samples were aqueous solutions of a dye, sunset yellow, which has a large molar absorption coefficient (ε=22400) at the excitation wavelength of 488.0 nm. Different concentrations were prepared by the stepwise dilution of a stock solution.

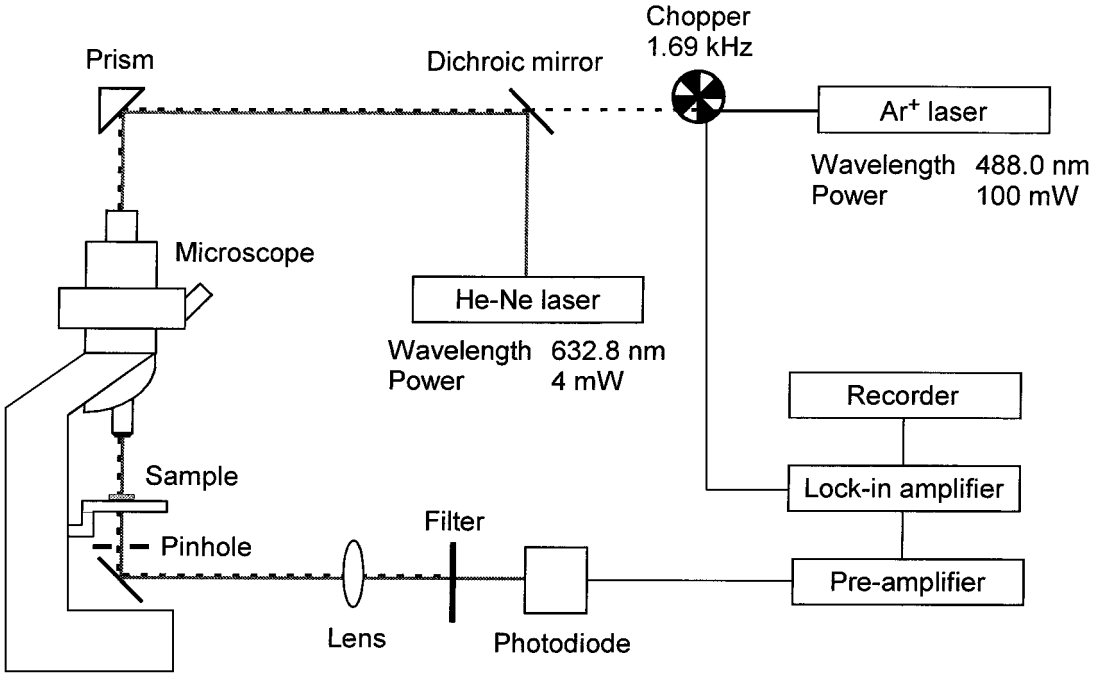

Fig. 2 Schematic diagram of the experimental setup.

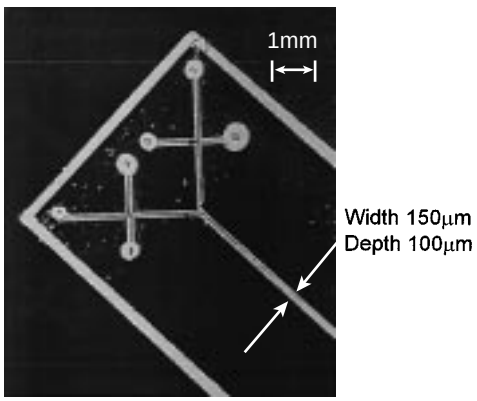

Fig. 3 Photograph of the microchip.

Results and Discussion

The glass chip was prepared by laser microfabrication, and is shown in Fig. 3. The details of the fabrication are reported elsewhere.[28] The chip was manufactured by laminating three artificial quartz substrates. In the middle and top substrates, the microchannel and holes were fabricated by a laser digging method. The thickness of the middle substrate was 100 μm and the width of the pierced channel pattern was 150 μm; the microchannel built into the chip was 150 μm wide and 100 μm deep. The reservoir holes in the top substrate were 1 mm in diameter. After the liquid sample was dropped onto the reservoirs, it flowed into the channel by capillary action; then, the glass chip was set on the sample stage of the thermal-lens microscope. The focal points of the coaxial beams were controlled in the center of the microchannel by moving the sample stage. Then, the dye sample was detected using the thermal-lens microscope.

First, the thermal-lens signal of the sample in the microchannel was evaluated by comparing it to the signal from a sample in a liquid micro space between a slide glass and a cover glass, which had roughly the same depth but much wider space. The respective signals are shown in Figs. 4(a) and 4(b). The signals arose during irradiation of the probe and excitation beams, and disappeared after cutting the excitation beam. As shown in Fig. 4(a), the thermal lens signal was very stable in its intensity; however, in Fig. 4(b) it shows a low-frequency fluctuation. We thought it reasonable to attribute this to convection flow of the sample solution. Once the thermal-lens formed, a temperature distribution occurred within the sample solution, and this would cause convection flow. When dye molecules in the microchannel absorb light, a large portion of the sample solution would be at almost the same temperature, because of the small volume of the sample solution. Furthermore, the motion of fluids in the microchannel would be restricted by the small space. Thus, the convection flow would be suppressed. By contrast, in the case of the micro space, there was a virtually infinite space in the horizontal direction, and the sample had a large degree of freedom in its mobility; therefore, convection easily occurred. This flow may randomly displace the location of the lens or change the locus of the probe laser, which would lead to fluctuation in signal intensity. Other groups have also investigated the effect of convection flow on the thermal-lens signal.[29,30]

Next, we measured a sample solution (5 μM) and water to estimate the absolute number of molecules

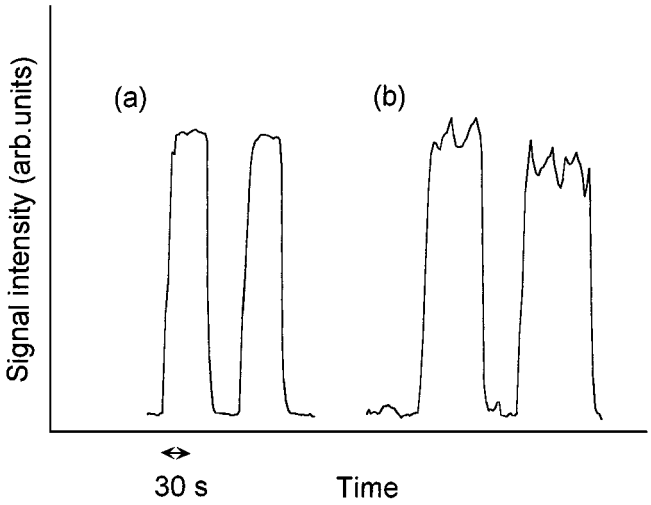

Fig. 4 Comparison of the thermal-lens signals from (a) a microchannel and (b) a micro space.

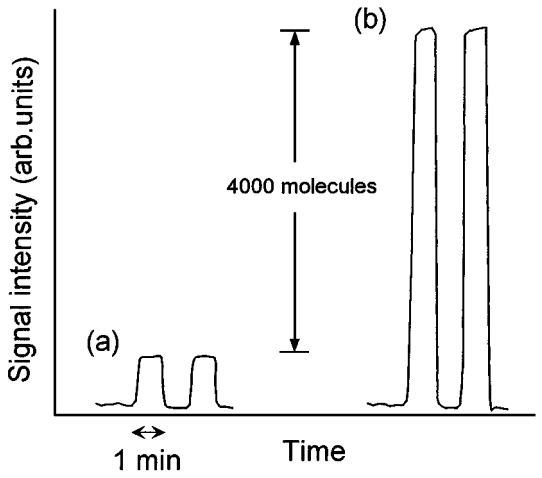

Fig. 5 Determination of dye molecules using the thermal-lens microscope. The difference in signal intensity between water (background) and the sample solution corresponded to the signal of the sample. The thermal-lens signals were obtained from water (a) and sample solution (b). The concentration of dye solution was 5 μM. The number of detected molecules was about 4000.

detected using the TLM. Hereafter, the excitation beam power just below the objective lens was 25 mW, the probe beam was 0.5 mW, and the chopping frequency was 1.69 kHz giving the best signal-to-noise ratio (*S/N*) under the present experimental conditions. The results are shown in Fig. 5. The difference in the signal intensity corresponded to the signal of the sample. Considering that the numerical aperture (N.A.) of the objective lens was 0.80 and the wavelength of the excitation beam was 488 nm, we calculated the volume of the detection area that corresponded to the confocal volume according to the thermal-lens theory, as 1.3 fl. Then, the number of detected molecules was estimated to be about 4000.

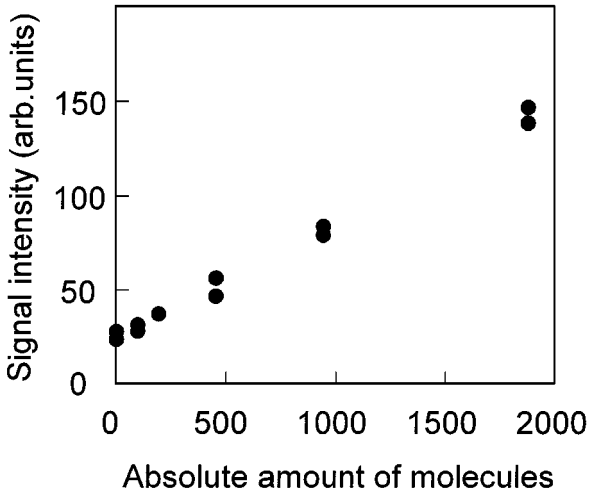

Fig. 6 Calibration curve of dye solution.

From this result, we tried to detect 0.1 – 2 μM of the dye molecules in water. The calibration curve is shown in Fig. 6. The signal showed good linearity in this concentration range. Considering the signal-contributing volume, we obtained a value of 0.13 – 2.6 zmol dye molecules as introduced into the microchannel. From this figure, the determination limit was estimated to be 160 ymol (*ca.* 100 molecules) by doubling the standard deviation. The detection limit was obtained as the number of molecules, where *S/N* was 2, and was 85 ymol (*ca.* 50 molecules). According to the nature of thermal-lens spectroscopy, some kinds of organic solvents can enhance the sensitivity as much as 40-times that in water.[27] Furthermore, ultratrace levels of molecules, a few molecules, can be detected.[31] This high sensitivity of the thermal-lens microscope method compares with that of the laser-induced fluorescence method, and is much larger than that of the absorption method.

Based on the results and discussion above, we concluded that TLM is one of the most promising methods for ultrasensitive detection in the ICL.

We have integrated an ultrasensitive detection system on a quartz glass chip using the thermal-lens microscope. Because the convection flow of a sample in a microchannel was restricted, the thermal-lens measurement was more advantageous than in a micro space. A sub-zepto-to-zeptomole level of the dye molecules was determined, and the determination limit was estimated to be 160 ymol. Even non-fluorescent molecules could be detected with our method, and detection sensitivity was as high as in the laser-induced fluorescence method. In particular, we expect that much more sensitive detection would be possible with some kinds of organic solvents.

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