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Temperature measurement of microfluids with high temporal resolution by laser-induced fluorescence †

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Abstract

Temperature of microfluidic system is greatly sensitive because of fast heat conduction and small heat capacity due to the scale effect. The purpose of this study is the development of a measurement system for the temperature field of liquids in a microfluidic device with high spatial- and temporal- resolution. Measurement method employed in this study is laser-induced fluorescence using fluorescein with the temperature dependence of fluorescent intensity. In order to measure the transient temperature field, an image-intensified high-speed camera was utilized. The signal-to-noise ratio can be improved by the time- or phase- averaging scheme. Applying the synchronization mechanism, phase-averaged temperature data with the time resolution of 500 μs can be obtained. Spatial resolution estimated from the Rayleigh limit was approximately 530 nm. The validity of the developed measurement system was confirmed by the experiments for the transient behavior of the liquid temperature undergoing the laser heating in the microfluidic device.

Keywords: Fluorescein; Laser-induced fluorescence; Microfluidics; Temperature measurement

1. Introduction

Microfluidic system has recently emerged owing to the development of micro- and nano- technology, and attracted considerable attention from biological, medical, chemical and engineering fields [1]. The integrated and minitualized fluidic device offers several advantages such as small sample volume, short reaction time, portability and potential for parallel operation. In microscale, heat and mass transport phenomena are quite different from those in macroscale due to the increase in the surface-to-volume ratio, so-called scale effect. Thus, the thermal behavior in the microfluidic device is governed by heat conduction rather than convection. In addition, the small heat capacity of the system results in a rapid thermal response. In capillary electrophoresis, a major tool for the separation of analyte ranging from small inorganic ions to large biomolecules, the Joule heat generated as the high voltage is imposed in a minute region can cause limitation on separation performance [2] or distortion of the migration velocity [3]. The temperature rise due to the Joule heating is also inevitable in electroosmosis [4, 5]. Recently, Shimakawa *et al*. investigated the potential of the temperature dependence of the liquid viscosity for the novel control method of the flow behavior in a microchannel [6]. Their approach involved the use of the scale effect of viscous force on transport phenomena [7]. Although they pointed out the validity of the optically-induced local property distribution for the control of microflow, the temperature field did not measured. It is important to understand the temperature distribution in a microchannel including the dynamic behavior.

The objective of the present study is to develop a measurement technique of the temperature of fluid in a microfluidic device with high temporal and spatial resolution by utilizing laser-induced fluorescence

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(LIF). The micro-LIF technique has a potential to obtain the fluid temperature with non-intrusive manner using a temperature-sensitive fluorescent dye. This study focuses on the measurement of the temperature distribution of liquid in a microfluidic device undergoing the laser heating.

2. Experimental setup

2.1 Temperature measurement system

Micro-LIF technique takes advantage of the temperature dependence of the fluorescent intensity of the dilute fluorophore added to the liquid. This method is capable of highly spatial-resolved measurement for the microfluidics with a totally optical approach. Fig. 1 shows the schematic of the measurement system for microfluidic temperature based on micro-LIF technique. It was assembled around an epi-fluorecent inverted microscope. As an illumination source, a continuous mercury lamp was employed. An exciter filter (ExF) extracts only the wavelength to excite the fluorescent dye from the light of the lamp. An air immersion objective lens having a magnification of 40 and NA of 0.95 was used to collect the fluorescence. After the passing an emitter filter (EmF) to select the fluorescent wavelength, the image was captured by a high-speed CMOS camera (512 × 512 pixels) through an image intensifier (gen-III). The frame rate for image acquisition determines the time resolution for the transient measurement of the temperature. The rate of CMOS camera of 2000 fps means the time resolution of 500 μs. A notch filter was inserted in front of the camera to eliminate only the wavelength of heating laser beam. Timings of laser heating and image acquisition are synchronized by a function generator (FG).

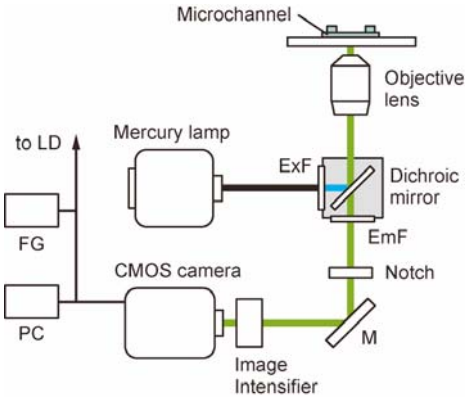

Fig. 1. Schematic of measurement system.

2.2 Microfluidic device

The microfluidic device in this study is a straight microchannel made of borosilicate glass with the rectangular cross-section of 500 μm in width, 50 μm in height and 50 mm in length. The difference in liquid surface levels between inlet and outlet reservoirs made of PMMA (polymethylmethacrylate) causes stable pressure-driven flow in the channel. Reynolds number based on a typical experimental condition in the present study was less than 0.1. The measurement section was 25 mm downstream from the inlet reservoir.

2.3 Working fluid

As a temperature-sensitive fluorescent dye, fluorescein ($C_{20}H_{12}O_5$) was employed. The peak wavelength for excitation and emission of fluorescein is 494 and 521 nm, respectively. Although rhodamine B is a popular fluorophore with stronger temperature dependence in the fluorescent intensity than fluorescein [8], a slight absorption in the wavelength of the heating laser in this study (635 nm) might affect the emission intensity. This is why fluorescein is used instead of rhodamine B. Tetra-borate buffer solution, hydrodynamically regarded as water, was used as the working fluid. The solution was selected to avoid the influence of pH variation in the liquid according to the temperature change, because fluorescein has strong pH dependence on the fluorescence intensity [9]. Considering that the pH variation of the liquid in the temperature range used in this study was within 0.3, the effect of the variation in the fluorescence due to the pH change on the measured temperature value was less than 1 K. In this experiment, concentration of fluorescein in the buffer solution was set at 0.1 mM.

In order to absorb the light of the heating beam, no-fluorescent absorption dye of Brilliant Blue FCF (BB; $C_{37}H_{34}N_2Na_2O_9S_3$) was added into the solution. Since BB has strong absorption band in the wavelength over 600 nm, the aqueous solution can be heated by the irradiation of the light with absorbing wavelength. The solution indicates no interference both with excitation and emission wavelength used for the temperature measurement.

2.4 Laser heating system

Fig. 2 indicates a schematic of the laser heating system. As a device to give the fluid a local temperature

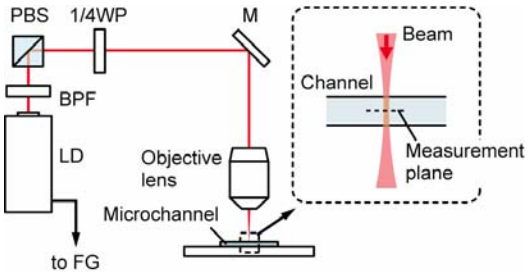

Fig. 2. Schematic of heating optical setup.

change, a compact diode laser (LD) with the wavelength of 635 nm was employed. The beam of LD passes through a polarization beam splitter (PBS) and a quarter wavelength plate (1/4WP). And then, the beam is focused by an objective lens (10×). The light intensity and irradiation timing are controlled by the FG used to trigger the camera for imaging. The focused beam diameter was set around from 30 to 40 μm. Generally, it is difficult for micro-LIF to measure the temperature field with nonuniformity in depth direction for observation, because this technique relies on the volume illumination and the planer detection of fluorescence based on the focal plane of the objective lens. The depth-average characteristic is unique only for micro-LIF technique [10, 11]. In this heating setup, the utilization of the low magnification objective lens for focusing enables a suitably constant beam waist within the channel. The measurement plane was set at 25 μm both from the upper and lower walls, center in heightwise direction; thus, temperature distortion or heat loss in heightwise direction was negligible.

3. Temperature measurement method

Micro-LIF is based on the temperature dependence of the fluorescent intensity of a fluorescent dye added in the fluid. The fluorescent intensity I can be presented as follows,

$$I = I_{ex} c \varepsilon \varphi(T) \tag{1}$$

where I_{ex} is the excitation light intensity, c is the concentration of fluorophore, ε is molar absorption coefficient and φ is the quantum efficiency as a function of temperature of the liquid. Although φ of fluorescein is sensitive to pH of the solution as well as temperature, pH is kept constant under the use of the buffer solution. The excitation light intensity should be constant in the measurement location, because the fluorescent intensity is dependent on the excitation light intensity. As a practical method, a calibration procedure is employed using the normalized fluorescent intensity which is divided by the reference fluorescence intensity I_{ref} at a reference temperature. In addition, the obtained fluorescent intensity includes the dark current noise or the stray light as the background noise. Here, the noise was reduced by subtracting the background intensity from the measured light intensity. Then the normalized fluorescent intensity with the background subtraction is calculated to obtain the ratio of the quantum efficiency as a function of the fluid temperature.

In order to measure the temperature field by the fluorescent intensity, a calibration experiment was conducted prior to the measurement of the concerned field. The microchannel was set on a thermostatically controlled copper plate connected to a circulating water bath, and then the fluid temperature was kept constant. A thin K-type thermocouple probe with the thickness of 40 μm was set close to the measurement area. Then the fluorescent images were acquired at seven temperature values ranging from 283 to 343 K when the fluorescent solution was fed into the microfluidic device. At each temperature for calibration, 10 images were averaged after the background subtraction for the calibration curve determination. The relationship between the normalized fluorescent intensity and fluid temperature was determined by curve fitting. Consequently, the temperature of the solution temperature can be measured by using the inverse function of fitted calibration function f_c as follows,

$$T = f_c^{-1}\left(\frac{I - I_d}{I_{ref} - I_d}\right) \tag{2}$$

where I_d is the background light intensity. In the present study, the reference temperature for normalization was 298 K. The calibration function f_c was given by least squares of the third-order polynomial approximation.

4. Results and discussion

4.1 Validation of temperature measurement

Fluorescent intensity images at each calibration temperature both in heating and cooling process were obtained. After that, the calibration curve was determined. Fig. 3 depicts the temperature dependence of

the fluorescent intensity and corresponding calibration curves at BB concentration of 0.1, 0.5 and 1.0 mM. In all cases, the fluorescent intensity had negative temperature dependence. The increase in the fluorescent intensity gradients according to the increase of dye concentration is confirmed. The temperature dependence of the fluorescence intensity of 0.1, 0.5 and 1.0 mM BB solution at the reference temperature of 298 K was -0.41, -0.51 and -0.68 %/K, respectively. The higher temperature dependence at higher BB concentration implies an overlap in the absorption band of BB and the emission band of fluorescein. The slight decay of longer wavelength in fluorescent spectrum due to the absorption by BB was recognized by using a spectroscope. Therefore, there is a need to use each calibration function for each BB concentration. At the fluorescence images in heating and cooling processes at 1 mM BB solution, the difference from the fitting curve was within 0.3 %; this difference corresponds to the uncertainty in the measured temperature of 0.5 K. In addition, the difference of measured temperature from the calibration curve among typical 9 areas (center and 8 surrounding regions near the ends) in a single image was less than 0.5 K throughout the calibrated temperature range. This fact indicates the uniformity in the measurement of the temperature field.

The spatial resolution in the experimental system determined from the Rayleigh limit derived from the collection wavelength and NA of the objective lens was estimated to be 530 nm. Although this is highly spatial-resolved to measure the temperature field in the microfluidic system, the signal of the single pixel in an image is so noisy that the accurate temperature information is difficult to be obtained only from it. There are two ways to remove the fluctuation of the pixel data and to increase the signal-to-noise ratio; spatial- and phase- averaging processing. Considering the repeatability of the phenomena in microscale, the phase averaging processing over several series of experiments in the same conditions was employed in the present study. Here, the noise reduction of averaged images acquired by the image-intensified high-speed camera was evaluated. Fig. 4 shows the effect of noise reduction by the averaging procedure. The RMS (root mean square) value of all pixels in the averaged image was drastically decreased as the average number was increased. The reduction rate reached 80 % in the average number of 30. Since the RMS value of 0.37 % was equivalent to the temperature fluctuation of 0.5 K, the average number of 30 was selected for the temperature measurement in this study. The phase-averaging method is also effective in keeping the high time resolution for measurement. Consequently, temperature measurement with the spatial resolution of 530 nm and the temporal one of 500 μs can be achieved in the present system.

Prior to the measurement of the temperature field in a microchannel with the laser heating, the temperature of microfluids in a microchannel with gradual temperature gradient was conducted. The temperature gradient was imposed by two Peltier elements set at inlet and outlet. One was heated and the other was cooled. The temperature at three positions, 9, 18 and 26 mm downstream from the inlet, were measured by micro-LIF and thermocouples attached near the each measurement location for comparison. The results are

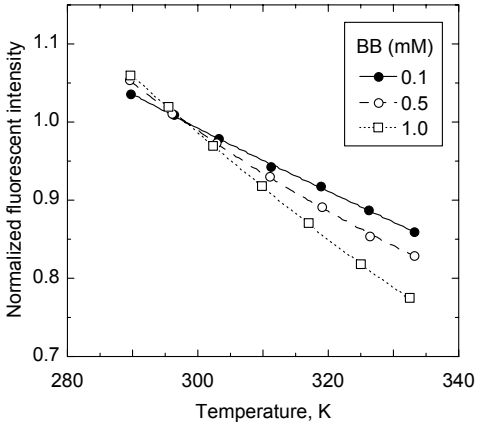

Fig. 3. Calibration curves between the corrected fluorescent intensity and the fluid temperature with the concentration of fluorescein of 0.1 mM.

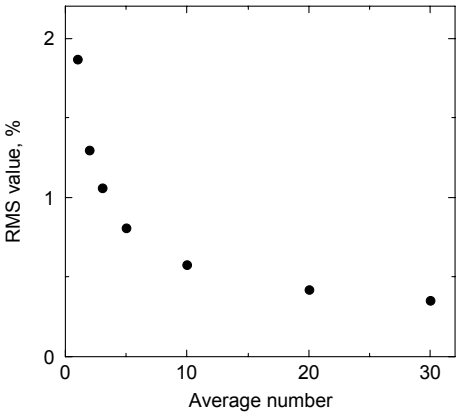

Fig. 4. Relationship between averaging number of fluorescent images by an image-intensified CMOS camera and RMS value of all pixels in the averaged image.

shown in Fig. 5. The measured value by micro-LIF was the spatially averaged value over 100 × 100 pixels, and the error bar depicted the standard deviation. Both results reflected the entire tendency of the temperature gradient. The measured values by micro-LIF was higher than those obtained by thermocouple. This is attributed to the difference in the measurement positions. Even though the measurement positions were close each other, the thermocouples were set at outside of the microchannel. Thus, there could be inherent temperature difference in those positions. Considering the inherent temperature difference due to the difference in location, both results indicate good agreement.

4.2 Temperature field in the microchannel under local heating

A preliminary measurement of the temperature was performed during the irradiation of heating laser into the working fluids without BB. As a result, no influence of the LD light on the fluid temperature was confirmed. Then, BB of the concentration of 1 mM was added to the buffer solution. The temperature field under the LD irradiation is shown in Fig. 6. X and y are streamwise and spanwise direction, repectively. The bulk velocity was approximately 1 mm/s. The high temperature region around the center corresponds to the irradiation area of the LD beam. This result indicates that the liquid temperature rise is caused by the laser heating.

Fig. 7 depicts the temperature profiles in the streamwise direction with several irradiating conditions of the laser power. The spanwise position was at the center of the laser spot. Each plot presented in the figure is the averaged value over 5 pixels. The bulk velocity was set at around 1 mm/s, same as in Fig. 6. The temperature change was nearly proportional to the irradiating light intensity at each position. The relaxation of the temperature rise toward downstream was gentle rather than upstream. This is considered to be the influence of the convection. The contribution of heat convection to downstream and heat conduction to the surrounding liquid on the temperature field can be evaluated by Peclet number defined as follow.

$$\mathrm{Pe} = \frac{\rho c_p L U}{\lambda} \tag{3}$$

where ρ is density, c_p is specific heat, L is the characteristic length, U is the characteristic flow velocity and λ is thermal conductivity of fluids. Peclet number based on the thermophysical properties of water, the height of the channel and the typical velocity in this

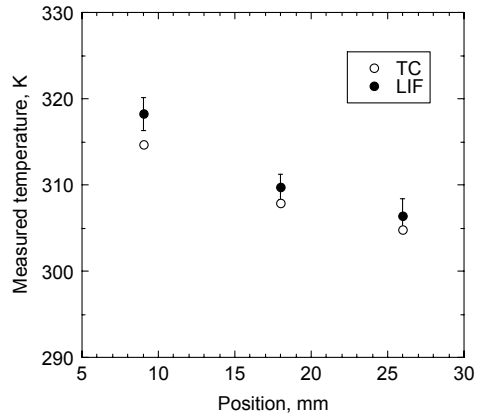

Fig. 5. Comparison of the measured temperature by micro-LIF and thermocouple.

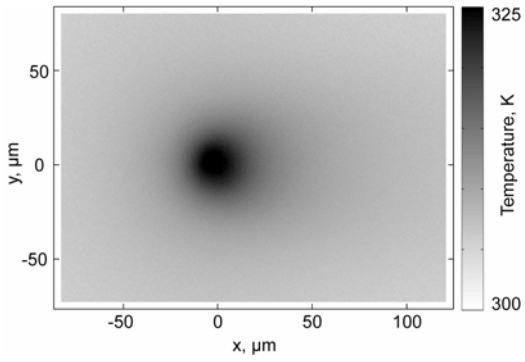

Fig. 6. Temperature distribution of the liquid in microchannel with the laser heating measured by micro-LIF. Flow occurs from left to right with the velocity of 1 mm/s.

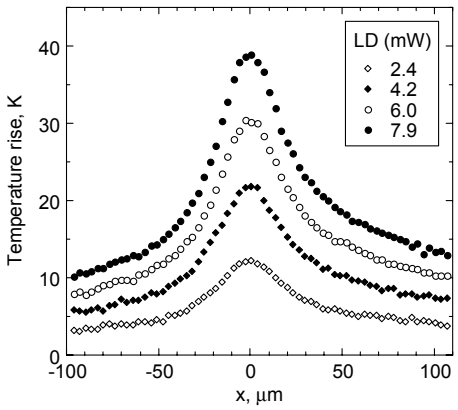

Fig. 7. Temperature distributions in streamwise direction. Spanwise position is at the center of focused spot.

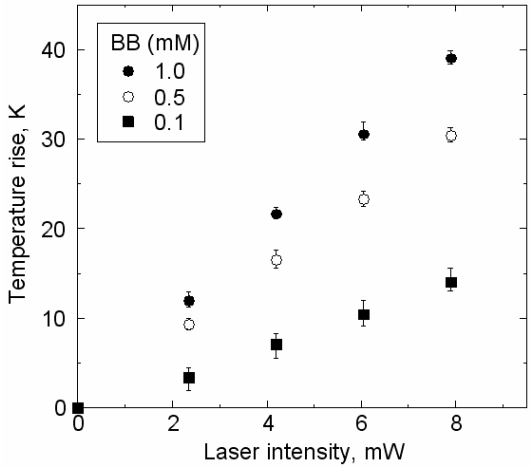

Fig. 8. Peak temperature values under several concentrations of absorption dye and irradiating laser intensities.

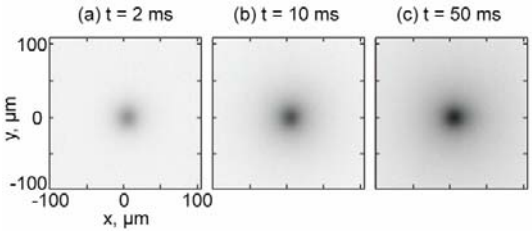

Fig. 9. Temperature fields under the laser heating at (a) 2, (b) 10, and (c) 50 ms after the irradiation. Flow occurs from left to right.

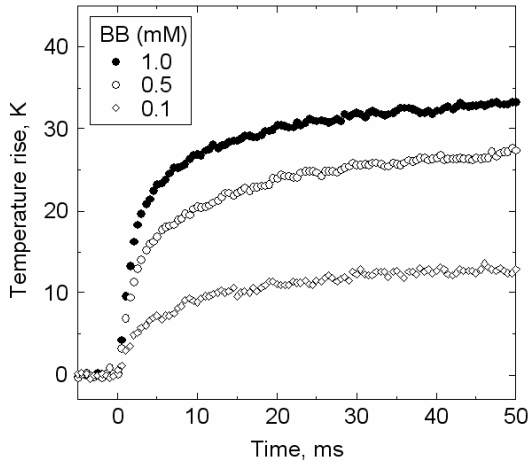

Fig. 10. Measurement results of transient response in the fluid temperature under the laser heating.

experiment was estimated to be about 0.2. In this low Pe number region, it can be said that the thermal behavior in the microchannel is dominated by the heat conduction rather than convection.

Fig. 8 indicates the relationship between the peak temperature and the irradiating light intensity at different BB concentrations. The peak temperature was averaged value over 5 × 5 pixels around the focused spot. The temperature value was linearly increased as the laser power is increased. The increase of BB concentration causes the elevation of the temperature rise.

4.3 Measurement of transient temperature field

The transient temperature field was measured by phase-averaging processing when the laser started a step heating. The frame rate for image acquisition of 2000 fps means the time resolution of this experiment of 500 μs. The light intensity was 8.0 mW, and the bulk velocity was around 1 mm/s. The temperature fields at 2, 10 and 50 ms are depicted in Fig. 9. Also, the measurement results of the time evolution in the peak temperature with the BB concentration of 0.1, 0.5 and 1.0 mM is shown in Fig. 10. After the laser was irradiated, the fluid temperature immediately rose and gradually converged to the certain value. In Figs. 9(a) and (b), the effect of convection on the temperature field were barely recognized, since the travel distance of the fluid in this temporal range was less than the beam size. An influence of the fluid flow as heat convection could be observed in the temperature distribution in Fig. 9(c). The time constants in the transient responses of the peak temperature in Fig. 10 were calculated to be approximately 10 ms, regardless of the BB concentration. This result is reasonable considering the similarity in the thermal behavior mainly governed by heat conduction.

4.4 Microflow behavior with local temperature distribution

Under the local temperature distribution by the focused laser beam, the corresponding distribution of the fluid properties can induce the change in flow structure [6]. Fig. 11 indicates the temperature field and corresponding velocity profiles in spanwise direction at the center of heating spot in streamwise direction. The velocity measurement was performed by micro-PIV (particle image velocimetry) technique [12]. The microchannel with the width of 200 μm was used in this experiment. The streamwise velocity at the focused spot was increased by about 10 %. This variation in the flow structure is caused owing to the local viscosity distribution with the temperature dependence. This property-driven particular phenome-

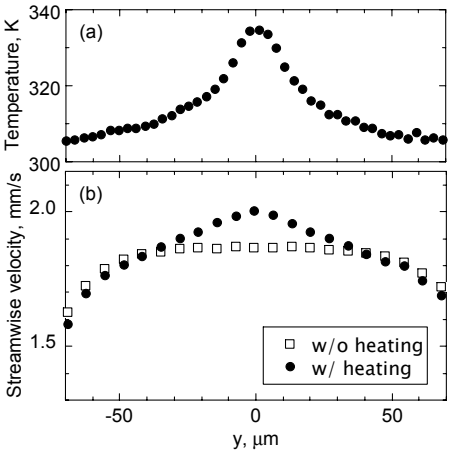

Fig. 11. (a) Temperature and (b) streamwise velocity profiles in spanwise direction under the laser heating. Local viscosity distribution causes the change in microflow structure.

non is unique especially in the microscale thermo-fluidic systems. The measurement system of the fluid temperature field with high temporal and spatial resolution can reveal the relationship between thermal and fluid behavior in the microfluidic device.

5. Conclusions

A system to measure the temperature of the fluid in the microfluidic device has been developed. Adopting the phase-averaging technique, the system could keep the high spatial and temporal resolution without reduction of signal-to-noise ratio; 530 nm and 500 μs in the present system were achieved. It is confirmed that the temperature rise is proportional to the concentration of absorption dye and the irradiating light intensity. In addition, measurement results of the temperature field indicated the heat conduction dominated the thermal behavior in the microfluidic system. The temperature field of the liquid in the microchannel undergoing a fast temperature change by the laser heating with the time constant of 10 ms could be measured by using developed experimental system.

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