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MICROSCOPIC FLUID TEMPERATURE MEASUREMENTS USING FLUORESCENCE POLARIZATION METHOD

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ABSTRACT

A fluid temperature measurement method in microscopic scale using fluorescence polarization is described in this study. The present method has the advantages in not only noncontact but also markedly reducing the influences of solution pH and quenching on the measured fluid temperature, compared to other methods including LIF (laser induced fluorescence) method. In the case of a fluid at rest, the fluorescence intensity varied with pH and linearly decreased with the elapsed time, while the polarization degree remained nearly constant. The polarization degree showed a good correlation with the fluid viscosity and temperature that corresponded to the tendency of the analytical results. The microchannel flows case also showed a good correlation between the fluid temperature and the fluorescence polarization degree was observed, which was similar to the stationary fluid case. These results affirmed the feasibility of our method using fluorescence polarization for fluid temperature measurement.

NOMENCLATURE

C	concentration of fluorescent molecules
I	fluorescence intensity
I_0	flux of the excitation light
$I_\perp$	fluorescence intensity of the perpendicular component
$I_{//}$	fluorescence intensity of the parallel component
P	polarization degree
t	time [s]
T	temperature [°C, K]
V	volume of the molecule [m^3]
k_B	Boltzmann constant [JK^{-1}]
ε	absorption coefficient

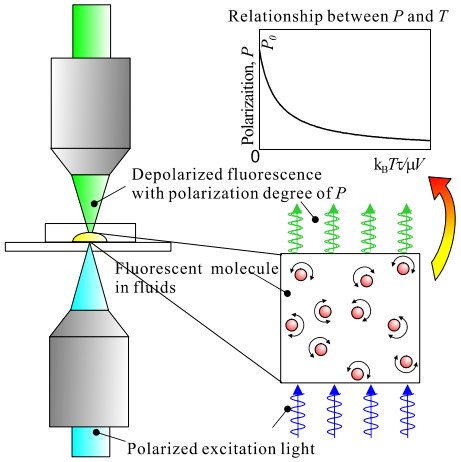

FIGURE 1. SCHEMATIC DIAGRAM OF THE MEASUREMENT PHYSICS USING POLARIZATION MEASUREMENT.

ϕ	quantum efficiency
λ	wavelength [m]
μ	fluid viscosity [Pa·s]
τ	fluorescence life time [s]
ξ	angle between the absorption moment and polarization direction

INTRODUCTION

Fluid temperature control in microchannels is an important issue to provide a suitable environment for cells and DNA, and to control the chemical reactions in the fields of medicine and chemistry. In order to control the fluid temperature in the

microchannel, a method that can measure the fluid temperature accurately in microscopic scale is very important.

There are currently several methods that are typically used to measure the fluid temperature in microchannels. Among these, thermocouples [1,2] and resistance thermometer [3] are relatively simple and low-cost to carry out a point measurement of the fluid and wall temperatures. In these measurements, however, a probe must be inserted in the channel that may affect the fluid motion or the fluid temperature. Further, the influence of the heat capacity of the probes becomes more apparent in micro-scale measurement that leads to a deterioration of the measurement accuracy and resolution.

On the other hand, laser induced fluorescence (LIF) method [4] derives the fluid temperature by measuring the fluorescence intensity of the fluorescent molecules mixed in the fluid. It does not require an insertion of mechanical probes in the fluid, which means LIF can be applied as a noncontact measurement. Another advantage compared with the thermocouples is its convenience in making a two-dimensional measurement. The measurement principle of LIF method is the quenching effect of the temperature on the fluorescence intensity, i.e. the fluorescence intensity decreases as the fluid temperature increases. The fluorescence intensity, however, is also influenced largely by the fluid pH, ionic concentration and measurement time. It is, therefore, difficult to subtract these effects from the temperature measurement during the calibration experiment. Several methods have been suggested to tackle these problems, such as using two types of fluorescent molecules and exposing them under excitation lights with different wavelength [5,6]. These methods however, require complicate calibration measurements.

In this study, a fluid temperature measurement method using fluorescence polarization is described. Figure 1 shows the schematic diagram of the measurement method. The fluorescent molecules are mixed in the working fluid, and are exposed to a linearly polarized light. The emitted fluorescence from the molecules is also polarized in this case. However, having the molecule experiencing the Brownian rotation, a partial depolarization of the fluorescence will occur. In this case, the polarization degree of the fluorescence becomes a function of the molecule volume, fluid viscosity, and the fluid temperature. If a molecule with appropriate fluorescence property and molecular size is chosen, the fluid temperature can be obtained by measuring the polarization degree of the suspended fluorescent molecules. The advantage of this method is that the polarization degree will not be influenced by the fluorescence intensity, namely the fluid pH and other quenching factors.

Measurements are carried out for the cases of stationary fluid in a reservoir and flowing fluids in a microchannel. In the stationary fluid case, the effects of the fluid pH, elapsed time, and fluid temperature on the fluorescence polarization degree are examined. Measurement is then carried out for microchannel flows to evaluate the relationship between the fluid temperature and polarization degree in order to verify the feasibility and performance of the present method.

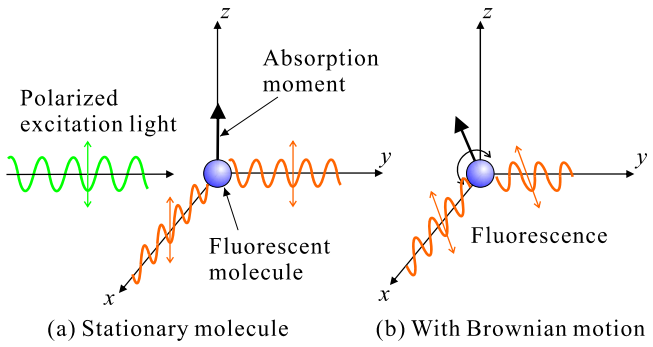

FIGURE 2. POLARIZATION DIRECTION OF THE EXCITATION LIGHT AND FLUORESCENCE OF THE MOLECULES AT STATIC AND BROWNIAN MOTIONS.

MEASUREMENT PHYSICS

As mentioned in the previous section, the present method measures the polarization degree of the fluorescence emitted from the molecules. The polarization direction of the fluorescence shows a strong relation with the polarization direction of the excitation light and Brownian motion of the molecules. The fundamental principles of the measurement method will be described in this section.

Figure 2 shows the relationship between the polarization directions of the excitation light, fluorescence, and absorption moment of the fluorescent molecule. As shown in Fig. 2(a), we now consider a fluorescent molecule, of which the absorption moment direction is parallel to the z axis and the position located at the origin, exposed to a linearly polarized excitation light. The absorption degree of the molecule to the excitation light depends on the angle between the absorption moment and the polarization direction of the excitation light, ξ. As ξ increases, this absorption rate decreases in the rate of $\cos^2\xi$. When the excitation light is polarized in the x direction, the molecule will not be excited. On the other hand, when the excitation light is polarized in the z direction, the absorption rate of the molecule becomes maximum (Fig. 2(a)).

The emitted fluorescence is also polarized in the same direction with the absorption moment. In other words, if an infinite number of fluorescent molecules randomly positioned are exposed to a linearly polarized excitation light, the molecules with the absorption moment parallel to the excitation light are stochastically excited. The fluorescence observed from these molecules is, therefore, mainly polarized in the same direction with the excitation light.

When the molecules are suspended in the fluid, the rotation of the molecule due to the Brownian rotation significantly influences the polarization direction of the fluorescence in addition to the effects of angle ξ. That is, since the molecule rotates during the period of the fluorescence emission, the polarization direction of the fluorescence shows

some variance with the direction of the excitation light (See Fig. 2(b)). This is the depolarization of the fluorescence.

To consider the relationship between the depolarization degree and other parameters, a variable called the polarization degree P is first defined as follows.

$$P = \frac{I_{//} - I_{\perp}}{I_{//} + I_{\perp}} \tag{1}$$

$I_{//}$ and $I_{\perp}$ represent the fluorescence intensities of the components that are parallel and perpendicular to the polarization direction of the excitation light, respectively. Perrin [7] and Weber [8] have theoretically derived Eqn. (2) to express the characteristic of P of a fluorescent molecule with Brownian motion.

$$\left(\frac{1}{P} - \frac{1}{3}\right) = \left(\frac{1}{P_0} - \frac{1}{3}\right)\left(1 + \frac{k_B T}{\mu V}\tau\right) \tag{2}$$

P_0 is the polarization degree of the molecule in steady state (without rotation). k_B is the Boltzmann constant. μ and T are the viscosity and temperature of the fluid, respectively. τ and V are the fluorescence relaxation time and the volume of the molecule, respectively. As one can see in Eqn. (2), the reciprocal number of P shows a linear relation with the T/μ. Therefore, if the molecular volume remains constant during the measurement, the fluid temperature can be obtained by measuring the polarization degree of the fluorescence. The present measurement is based on this physics.

The total fluorescence intensity I can be influenced by various factors. For example, I can be presented by Eqn. (2).

$$I = I_0 C \phi \varepsilon \tag{2}$$

I_0 is the intensity of the excitation light and C is the concentration of the florescent molecules. ϕ is the quantum efficiency, and ε is the absorption coefficient presenting the rate that the excitation light is absorbed while it passes through a unit length of solution.

In addition to these effects, the decrease of fluorescence intensity, so called a quenching effect, occurs attributed to various reasons [9]. For example, when the fluid temperature increases, the molecular motion in the solution becomes active. In this case, the fluorescent molecules collide with other molecules that leads to a decay of the molecular energy, i.e. the decrease of fluorescence intensity. The change of the fluid temperature also produces a change in the vibration motion of the molecule, hence, the fluorescence intensity. Fluid temperature measurement using the laser induced fluorescence (LIF) method employs this relationship between the fluid temperature and fluorescence intensity.

The fluorescence intensity is, however, also influenced by the pH of the solution, particularly with molecules including

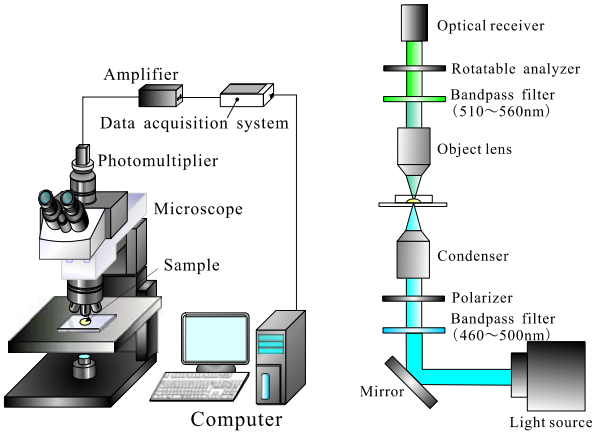

FIGURE 3. SCHEMATIC VIEW OF THE EXPERIMENTAL APPARATUS AND OPTICAL SYSTEM.

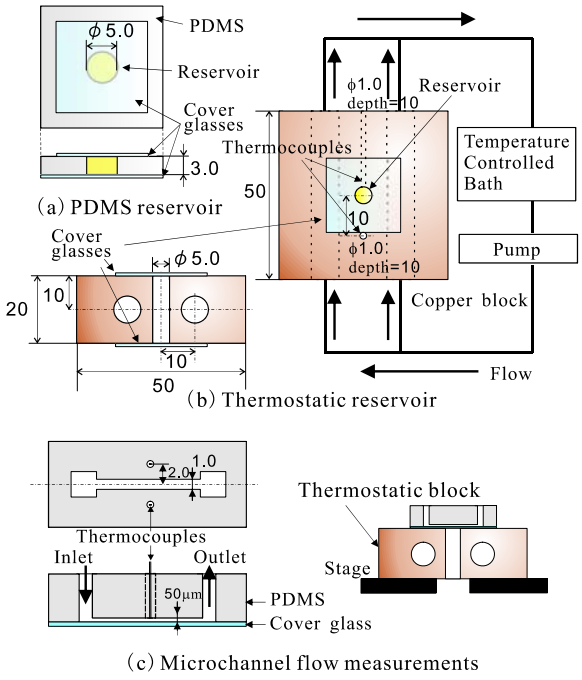

FIGURE 4. SCHEMATIC VIEW OF THE RESERVOIR FOR STATIONARY FLUID MEASUREMENTS AND MICROCHANNEL.

acidic. Oxygen quenching that is attributed to the oxygen molecules, which are solved in the fluid, colliding with the fluorescence molecules is also another typical quenching effect. These other effects will inevitably influence the performance of the LIF measurements, and are not preferable. Therefore, several methods have been proposed in order to reduce the influence of these quenching effects, or to subtract such effects from the original data.

On the other hand, the present method measures the polarization degree, P. As shown in Eqn. (1), P is a value normalized by the value $I_{//}+I_{\perp}$ that presents the total fluorescence intensity. P is, therefore, not influenced by the variation of the fluorescence intensity. This means that the

previously mentioned quenching effects can be ignored in the present method.

EXPERIMENTAL PROCEDURE

Figure 3(a) shows the schematic view of the experimental apparatus used to measure the polarization degree *P*. Figure 3(b) shows the schematic diagram of the optical system. The apparatus is composed of a microscope (Olympus; BX-51), photomultiplier (Hamamatsu; C7319) and data acquisition equipment (National Instruments; USB-9219). A halogen lamp is used as the light source. The light passes the band-pass filter (Semrock; FF01-482/35-25, λ=482±18nm) and a condenser, in which a polarizer is embedded, and is emitted to the test section as the excitation light. The fluorescence is measured by the photomultiplier through an objective lens (Olympus; UMPlanFL, ×10, *NA*=0.30, working distance=10.1mm), a band-pass filter (Semrock; FF01-536, λ=536±20nm) and a rotatable analyzer (Olympus; U-AN360-3).

The polarization degree, *P*, was measured by the following procedure. The angle of the polarizer installed in the excitation pathway was fixed. The angle of the analyzer was calibrated for the directions parallel and perpendicular to the polarization direction of the excitation light without inserting the test section in the optical pathway. For the fluorescence measurement, the components of the intensity that are parallel and perpendicular to the excitation polarization ($I_{//}$ and $I_{\perp}$) are measured by rotating the analyzer during the measurement. *P* is then obtained applying these values to Eqn. (1).

Measurements were made for two kinds of flow conditions: one is a fluid at rest and the other is the fluid flowing in a microchannel. Figure 4 shows the schematic diagrams of the test section used in these measurements. In Figs. 4(a) and (b) are shown the reservoirs prepared for the stationary fluid measurement. The reservoirs were 5mm diameter circular cylinders made of PDMS and cupper, respectively. For the cupper reservoir, water of constant temperature was supplied from a thermostat to the cupper block in order to maintain the wall and fluid temperature inside the reservoir constant. The fluorescence solution was sealed in the reservoir by attaching cover glasses to the top and bottom walls. Black paint was sprayed on the surface of the cylinder inner wall to reduce the reflections. K-type thermocouples were embedded in the cupper block at the locations depicted in Fig. 4(b) to measure the wall temperature.

Measurement for the microchannel flow was carried out using the test section shown in Fig. 4(c). The microchannel was made of PDMS (poly-dimethylsiloxane, Shin-Etsu Chemical Co. Ltd.; KE-106) and was fabricated using SU-8 (MicroChem Co.) as a casting mold. The PDMS channel, which was removed from the SU-8, was attached to a cover glass. The channel had a cross-section of 1mm width and 50μm height. Fluid was supplied from a syringe pump (Nihon Kohden; CFV-3200) to the channel with a flow rate of 0.5μl/min.

The microchannel was attached to the top wall of the cupper block with the channel center positioned at the center of the cylinder through which the excitation light was emitted. K-type thermocouples were embedded aside the microchannel at the locations illustrated in Fig. 4(c).

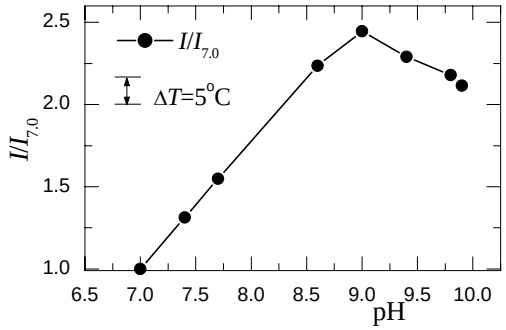

FIGURE 5. pH EFFECTS ON FLUORESCENCE INTENSITY, *I*.

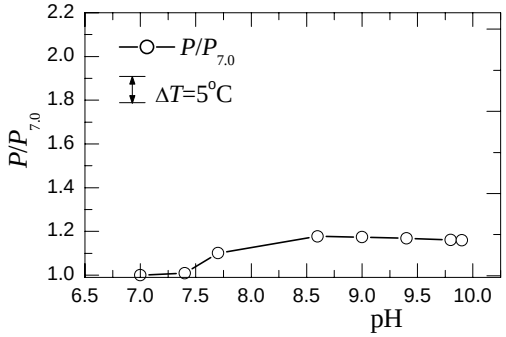

FIGURE 6. pH EFFECTS ON POLARIZATION DEGREE, *P*.

EXPERIMENTAL CONDITIONS

As mentioned in the above section, polarization measurement was made for the stationary fluid and the microchannel flow. The elapsed time during the measurement, fluid pH, and fluid temperature were varied in order to evaluate their influences on the polarization degree, *P*. For the stationary fluid measurement, the influence of the pH was measured using the PDMS reservoir shown in Fig. 4(a); a condition closer to the one used in practical applications. For the measurement to evaluate the effects of the elapsed time and fluid temperature, the cupper block type reservoir was used to control the temperature. For the microchannel flow measurement, the fluid temperature was varied using the microchannel attached to the cupper block as shown in Fig. 4(c).

Fluorescein-5-isothiocyanate (FITC) was used as the water soluble fluorescence molecule. The molecular weight of the FITC was MW=389. Considering the distribution of *P* against the variable $k_B T\tau/\mu V$ obtained from Eqn. (2), *P* reciprocally increases as the term $k_B T\tau/\mu V$ decreases. This indicates that a higher sensitivity is achieved in the region of larger *P* since the present method evaluates the temperature difference from the variation of *P*. Considering then that *P* decreases as $1/V$ decreases, a molecule with greater volume will enhance the measurement sensitivity. For this reason, casein-FITC-conjugated (ABD Bioquest; MW=13440, hereafter referred to

as C-FITC) is used in the measurement. Casein has a much larger molecular size than FITC, and is a protein that can be marked easily by the FITC.

Phosphate buffer saline (Nakarai tesque) was used as the solvent. C-FITC was mixed with the solvent at a concentration of 0.01g/l (7.4×10^{-7}mol/l). The pH of the solution was checked before measurement using a pH sensor (Horiba; B-212). Except the measurement evaluating the pH effect, the pH of the solution was kept as pH=9.18.

The sampling rate of the data acquisition system was 2Hz. 5 samples were measured continuously and was averaged for the $I_{//}$ and $I_{\perp}$ components by rotating the analyzer. This process was made alternatively 10 times, and the averaged value was taken as the result. From the $I_{//}$ and $I_{\perp}$ values, P was calculated using Eqn. (1).

RESULTS AND DISCUSSION

Figures 5 and 6 show how the fluid pH influences the fluorescence intensity I and the polarization degree P in the stationary fluid case. The results are normalized by $I_{7.0}$ and $P_{7.0}$ that present the I and P values of pH=7.0, respectively. $I/I_{7.0}$ shown in Fig. 5 takes a maximum value at pH≅9.0, and decreases as pH increases or decreases. This result shows the quenching effect of pH on the fluorescence of C-FITC. As one can see, the value at pH=9.0 is approximately 2.5 times larger than the value of pH=7.0, i.e. $I_{7.0}$.

On the other hand, as shown in Fig. 6, the value $P/P_{7.0}$ is nearly constant in the range of 7.0≤pH≤7.5, and then increases in the area of pH=7.5~8.5. At pH greater than 8.5, $P/P_{7.0}$ remains almost constant. The difference between the maximum and minimum values is 5.5%: a much smaller value compared to the $I/I_{7.0}$ case in the corresponding pH region. The range of the vertical axes of the graphs shown in Figs. 5 and 6 are adjusted so that the same variation in each graph will represent the same amount of temperature variation (the ranges corresponding to 5°C temperature difference in the measurement are depicted in each figure). Therefore, the difference in the variation in each graph will directly represent the variation in the temperature measurement. This shows that the variation attributed to the fluid pH can be markedly decreased in the polarization measurement that leads to higher resolution and accuracy of the temperature measurement.

Figures 7 and 8 show the influence of the measurement elapse time t on the I and P values. The results are normalized by $I_{t=0}$ and $P_{t=0}$, that present the values at the starting time of the measurement, respectively.

As shown in Fig. 7, $I/I_{t=0}$ gradually decreases as the measurement starts. At time t =110min, I shows 20% reduction compared with $I_{t=0}$. This is considered to be due to the quenching effects of various causes as described in the previous section. $P/P_{t=0}$ in Fig. 8, however, remains nearly constant during the measurement. Same as in Figs. 5 and 6, the range of the vertical axis in the two figures corresponds to the same range of temperature variation. In this case, the variations

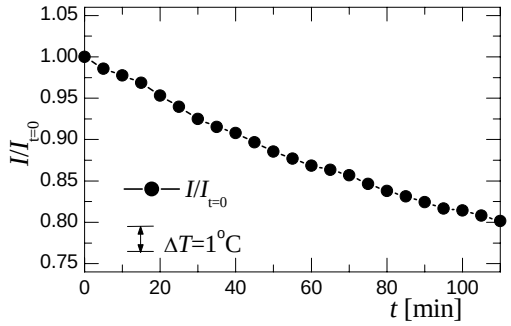

FIGURE 7. MEASUREMENT ELAPSED TIME EFFECTS ON FLUORESCENCE INTENSITY, *I*.

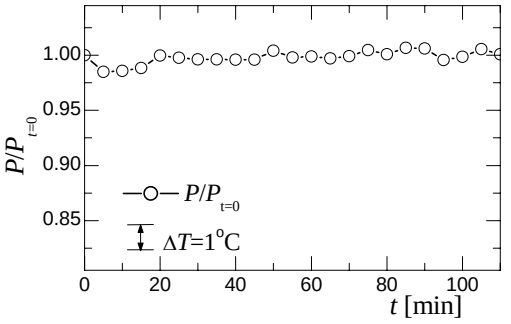

FIGURE 8. MEASUREMENT ELAPSED TIME EFFECTS ON POLARIZATION DEGREE, *P*.

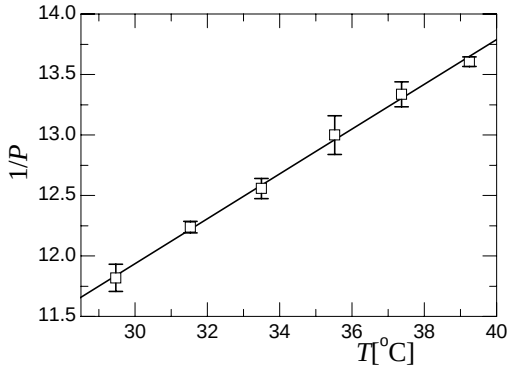

FIGURE 9. RELATION BETWEEN RECIPROCAL OF *P* AND FLUID TEMPERATURE *T* IN RESERVOIR.

during the period of 0≤ t ≤110min for $I/I_{t=0}$ and $P/P_{t=0}$ correspond to the temperature differences of 10°C and 1.4°C, respectively. The influence of time on the present method is, therefore, markedly small compared with the methods using the fluorescence intensity measurement.

Figure 9 shows the relation between the reciprocal of P and the fluid temperature in the reservoir T. The line drawn in the graph was derived on the basis of the least square mean approximation using all plotted data. A good linear correlation between $1/P$ and T is observed in the graph. The error bars shown in the figure represents the results of uncertainty analysis conducted on the basis of the ASME Performance Test

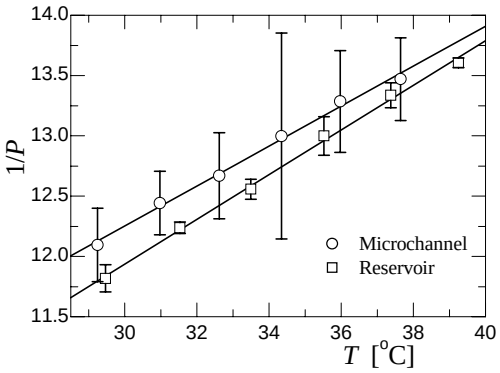

FIGURE 10. RELATION BETWEEN RECIPROCAL OF *P* AND FLUID TEMPERATURE *T* IN MICROCHANNEL.

Code [10]. As shown in the figure, the maximum error corresponds to the temperature difference of approximately 0.49°C.

Figure 10 shows the relation between 1/*P* and the temperature *T* of the fluid flowing in the microchannel. The results of the stationary fluid case are also shown in the figure for comparison. Same as the results shown in Fig. 9, 1/*P* linearly increases against *T*, and a good correlation is observed. This result affirms the feasibility of the present method for temperature measurement in microchannel using fluorescence polarization.

Comparing the results obtained from the stationary fluid in the reservoir (Fig. 9) and the flowing fluid in microchannel (Fig. 10), the results of the latter case show larger 1/*P* value and smaller slope in the least square mean line. This indicates lower measurement sensitivity. The reason for this is considered to be attributed to the optical quality of the PDMS microchannel wall. Since the fluorescence was measured through the PDMS, depolarization of the fluorescence might have occurred for some degree as the fluorescence transmits. This depolarization effect will be superimposed on that caused by the Brownian motion. Therefore, *P* is equally increased in the overall range and the measurement sensitivity decreases.

In addition to this, an apparent increase in the error is observed in the microchannel case. The reason for this is believed to be attributed to the decrease of the fluorescence intensity. As shown in Fig. 4(c), the microchannel was attached on the cupper block. The distance between the condenser and the working fluid with fluorescent molecules, therefore, increased that leads to a decrease of the intensity of the excitation light. Further, the volume of the microchannel was much smaller compared with the reservoir. Therefore, the number of the fluorescent molecules measured was much smaller the microchannel. For these reasons, the fluorescence intensity decreased and the influence of the error in the optical measurement increased in the microchannel case. These problems can be solved by adjusting the focal position of the illumination and measurement parts, or increasing the concentration of the fluorescence molecules.

CONCLUSIONS

A noncontact fluid temperature measurement in microscopic scale using fluorescence polarization was described. The effects of pH, elapsed time and fluid temperature on the measurement of fluid at rest and microchannel flow were discussed. A linear relation between the polarization degree and fluid temperature was observed for both cases. The pH and elapsed time had a considerable influence on the fluorescence intensity whereas the polarization degree was scarcely affected. These results showed the feasibility and advantage in using fluorescence polarization for fluid temperature measurement.

ACKNOWLEDGMENTS

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