Proceedings of the 4th International Forum on Heat Transfer, IFHT2016
November 2-4, 2016, Sendai, Japan

IFHT2016-1987

FLUID TEMPERATURE MEASUREMENT IN MICROCHANNELS USING FLUORESCENCE POLARIZATION METHOD

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ABSTRACT

A fluid temperature measurement technique based on fluorescence polarization is developed and applied to measure the two-dimensional temperature distributions in microchannel. The fluorescence depolarization due to the Brownian rotational motion of the fluorescent molecules is measured in this method and is converted to the fluid temperature. Since the fluorescence polarization degree is independent of fluorescence intensity, the fluorescence polarization is less affected by the quenching effects, which is one of the drawbacks of LIF (laser-induced fluorescence). Experiments were performed using a microchannel and fluorescent molecules solved in liquid. In order to examine the validity of the measurement, the effects of the fluid viscosity and temperature on the fluorescence polarization were measured. Further, linear temperature gradient was generated in the microchannel and measured by the present method. The results showed that as the fluid viscosity decreased and the temperature increased, the fluorescence depolarization increased. The results agreed well with the theoretical values and thermocouple measurements confirming the validity and the feasibility of the method.

KEY WORDS: Fluorescence polarization, Fluid temperature measurement, Microscopy, Microchannel, FITC

1. INTRODUCTION

Microscale temperature measurement is an important issue in micro-devices so called lab-on-a-chip and micro total analysis system (μ-TAS). For example, in biochemical reaction represented by the DNA hybridization process requires a temperature control and, therefore, measurement of an accuracy of $\pm$1°C [1].

The conventional techniques in measuring the fluid temperature in the microchannel are thermocouple [2], resistance thermometer [3], and laser-induced fluorescence (LIF) [4] methods. Thermocouples and resistance thermometers are relatively reliable, compact and low in cost. However, since these probes are intrusive and have a characteristic size relative larger than or equal to the microchannel, the spatial resolution is low. LIF, which is based on the temperature dependence of the fluorescence intensity, overcomes this limitation and allows us to obtain the fluid temperature with high spatial resolution. However, there are some issues in the measurement using LIF: the fluorescence intensity is affected by the variation of the excitation light intensity and quenching effects related to factors other than temperature.

To tackle these problems, a novel method measuring fluorescence polarization is proposed in this study. The depolarization degree of the fluorescence emitted from the fluorescent molecules, which are exposed to linearly polarized light, can be an index to measure the fluid temperature. In the present study, the relationship between the polarization degree and fluid temperature is measured for microchannel flow in order to validate and examine the feasibility of the measurement method.

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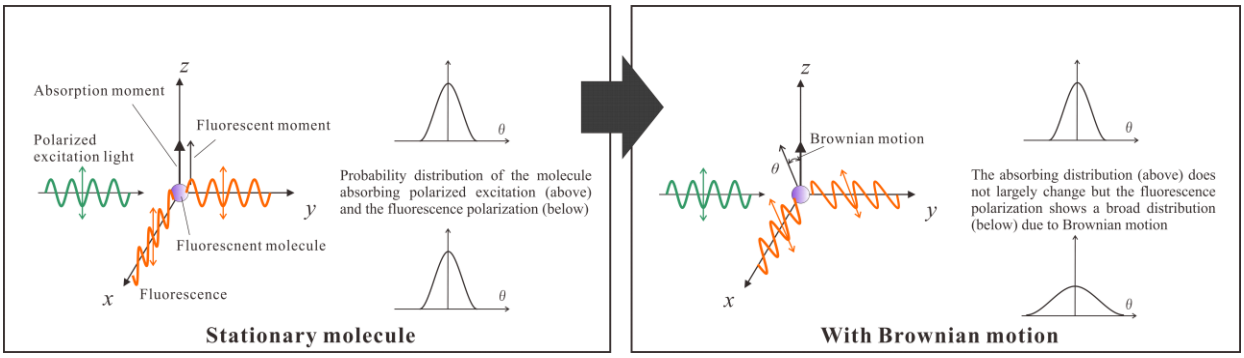


Fig. 1 Schematic of the relationship between the polarized excitation light and the polarization degree of the fluorescence in the cases of the molecules in the stationary state and the presence of Brownian motion. The probability density distribution shows the probability of the directions of the absorption and fluorescence moments.

2. PRINCIPLE

The physics of the temperature measurement method using the polarization degree of fluorescence is described in this section. Figure 1 shows the relationship between the polarization directions of the linearly polarized excitation light, absorption moment of the fluorescent molecule and fluorescence. Among the molecules exposed to the excitation light, those with the absorption moment having a component aligned parallel to the polarization direction of the excitation light are excited. The absorption degree of these molecules depends on the angle between these directions, θ, and can be expressed by the probability density function of absorption which is proportional to $\cos^2\theta$. The excited fluorescent molecules will then emit fluorescence polarized in the same direction with the absorption moment.

In the case of fluorescent molecules in stationary state without rotation, the fluorescence is polarized in the same direction with the excitation light with some variation owing to the probability density function mentioned previously. On the other hand, when the fluorescent molecules are dissolved in fluid, the rotational motion due to Brownian motion influences the polarization direction of the fluorescence. In this case, the excited molecules experience the rotational motion during the period of the excitation and emission, and the polarization direction of the fluorescence shows greater randomness compared with the fluorescence of molecules in stationary condition. Namely, depolarization is observed.

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

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

$I_{\parallel}$ and $I_{\perp}$ present the fluorescence intensities of the components that are parallel and perpendicular to the polarization direction of the excitation light, respectively. Perrin [5] and Weber [6] have theoretically derived Eq. (2) to express the characteristic of P of fluorescent molecules with Brownian motion.

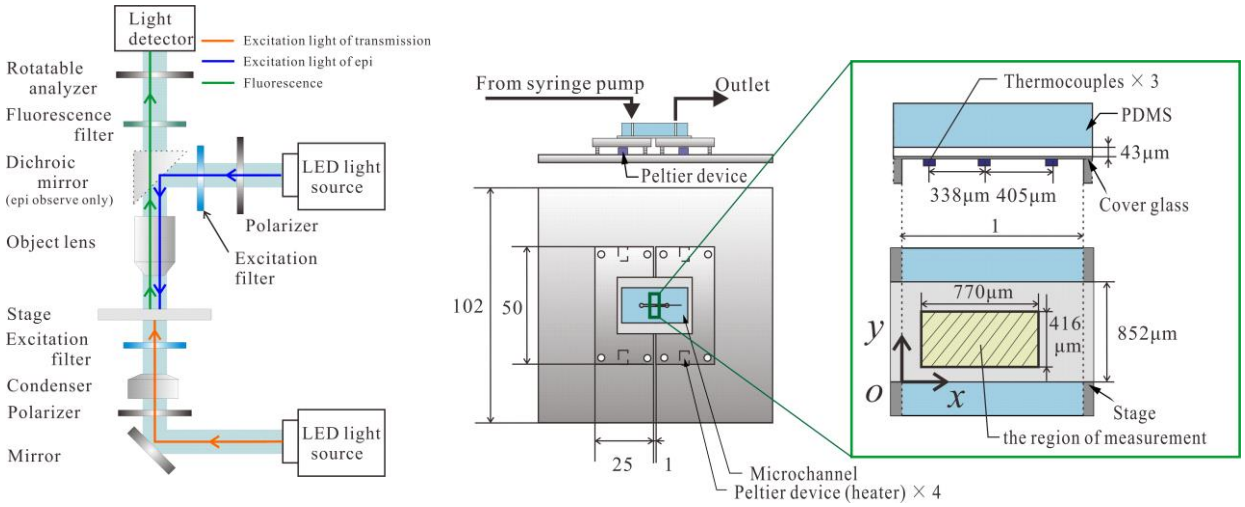
$$\left(\frac{1}{P} - \frac{1}{3}\right) = \left(\frac{1}{P_0} - \frac{1}{3}\right)\left(1 + \frac{k_B T}{\mu V}\tau\right) \tag{2}$$

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 volume of the molecule, respectively. As one can see in Eq. (2), the reciprocal number of P shows a linear relation with 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.

3. MEASUREMENT SYSTEM

Measurement was conducted by upright microscope (Olympus, BX51) and LED (Thorlabs, M490L3) light source. Figure 2(a) shows the schematic of optical system. To separate the excitation light and fluorescence, the fluorescence excitation filter (Semrock, FF01-482/35-25), dichroic mirror (Semrock, FF506-Di03-25x36), the fluorescence filter (Semrock, FF01-536/40-25) were placed in the optical path. Fluid viscosity effects on fluorescence polarization were measured by transmission observation. The excitation light from the LED was linearly polarized by the polarizing light condenser (Olympus, U-POC-2) and illuminated the fluid in the microchannel. The fluorescence through analyzer (Olympus, U-AN360P-2) was measured by photomultiplier (Hamamatsu Photonics, H5783-03). On the other hand, fluid temperature effects on the fluorescence polarization were measured by epi observation. The excitation light passed through polarizer (Olympus, U-PO3), and the fluorescence observed through rotatable polarizer (Olympus, LP40120) was received by high sensitivity camera (Andor, Zyla DV435) with an exposure time of 1s.

Figure 2(b) shows the microchannel fabricated by PDMS and the temperature control stage. The channel was a straight one with the height of 43μm and width of 850μm. The stage was made of aluminum and composed of a bottom plate and two top plates. The top plates were set parallel with 1mm spacing. Between the bottom plate and each top plate, two peltier devices were placed to control the stage temperature. Although not shown here, the plate temperature was measured by thermography (FLIR systems, ThermoCAM E65) and thermocouples when the stage was heated, and it was validated that the surface temperature of the stage was uniform. The microchannel was placed on the top plates with the channel direction set perpendicular to the gap of the plates. Three thermocouples were attached to the backside of the channel bottom wall (cover glass) equally spaced by approximately 300μm.



(a) Optical path of measurement (b) Microchannel and temperature control stage

Fig. 2 Schematics of the (a) optical path in the microscope and (b) straight microchannel and temperature control stage used for temperature measurement.

4. RESULTS AND DISCUSSION

4.1 The Viscosity Effects on Fluorescence Polarization

Fluid viscosity effects on the fluorescence polarization are discussed in this section to compare with the theoretical value for validation and to discuss the effects of the fluorescent probe used in the measurement. To change the fluid viscosity, glycerol (Nacalai Tesque, #17018-25) was mixed with water. Two fluorescent molecules, which have different volume, were examined. One is fluorescein isothiocyanate (FITC, MW=389: Nakalai Tesque, #V9E9989) and the other is Casein-FITC conjugated (C-FITC, MW=26000:

AAT Bioquest, #13440). FITC and C-FITC have different molecular weight. Their size are approximately 1nm and 10nm, respectively.

Figure 3 shows the relationship between the inverse value of fluid viscosity, $1/\mu$ and fluorescence polarization degree, P. The theoretical values represented by the solid line are derived from Eq. (2) under the condition shown in Table 1. In the case of FITC, P decreases as the fluid viscosity decreases. The Brownian motion becomes more vigorous as μ decreases and depolarization is observed. Comparing the experimental results with the theoretical ones, the two values agree reasonably well showing the validation of the present measurement.

In the case of C-FITC, P or the theoretical value is constant against μ. This shows that no rotational motion exists during the period of the excitation and emission. However, the experimental values decrease largely in the region approximately at $1/\mu$ = 50 $Pa^{-1}{\cdot}s^{-1}$. Although not shown here, the particle diameter was measured based on dynamic light scattering method (Otsuka electronics, ELSZ-2plus). The results showed that the diameter largely decreases at approximately $1/\mu$ = 50 $Pa^{-1}{\cdot}s^{-1}$. This result suggests the possibility that the FITC (or a part of C-FITC) detached from the casein at this condition. Since the isolated molecular or cluster is smaller than C-FITC, P is decreased in the region at approximately $1/\mu > 50$ $Pa^{-1}{\cdot}s^{-1}$.

Under the fluid viscosity condition of water, $1/\mu \cong$ 1000-1200 $Pa^{-1}{\cdot}s^{-1}$, P of C-FITC case shows greater value compared with that of FITC case. This shows a larger signal in the measurement. Therefore, C-FITC was used for the temperature measurement to increase the S/N ratio.

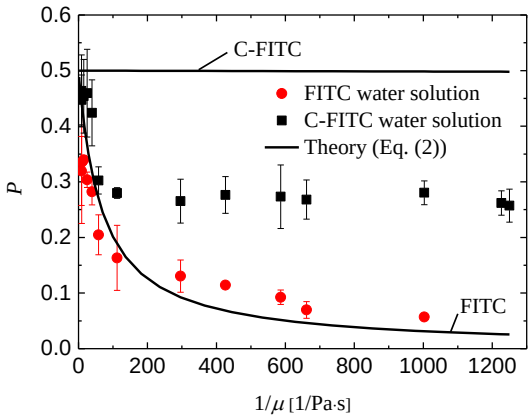


Fig. 3 Fluid viscosity μ effects on the fluorescence polarization P in the cases of FITC and C-FITC solutions. The solid lines present the theoretical values calculated by Eq. (2) applying the values shown in Table 1.

Table 1 Properties applied to Eq. (2) to calculate P of FITC and C-FITC shown in Fig. 3.

Properties	FITC	C-FITC
P_0	0.5	0.5
k_B	1.38×10^{-23} J/K	1.38×10^{-23} J/K
T	303 K	303 K
τ	4 ns	4 ns
d	1.08 nm	10 nm

4.2 Fluid Temperature Measurement

Calibration. Calibration was performed on the fluorescent polarization degree, P, of C-FITC 0.1wt% aqueous solution for fluid temperature in the temperature range from 28 to 38°C. This is a reasonable range considering the temperature applied to the biochemical assay. The temperature in the channel was kept constant at each

temperature condition. The fluorescence intensity and P distributions in the area of 416μm × 770μm shown in Fig. 2(b) was measured by the camera.

The spatial systematic error of the optical systems, $B(x, y)$, was calculated using the following formula and was subtracted from each image.

$$B(x,y)=\frac{1}{N}\sum_{i=1}^{N}(P_i(x,y)-\overline{P_i}) \tag{3}$$

$\overline{P_i}$ is a spatially averaged value of the ith image and N is the number of images.

Figure 4 shows the results of the calibration. The plots in the graph are (P – B) averaged over the area and of ten images. The straight line drawn in the figure was derived on the basis of the least square mean approximation. The error bars were calculated from ten images and they present the 95% confidence range for the uncertainty of each temperature condition. In the figure, 1/P increases linearly against the fluid temperature T with the relation presented as Eq. (4).

$$T=\frac{\frac{1}{P}-6.43}{0.324} \tag{4}$$

The accuracy of the present method was ±2.99°C. This empirical relation matches to Eq. (2) and confirms the validity of the present method.

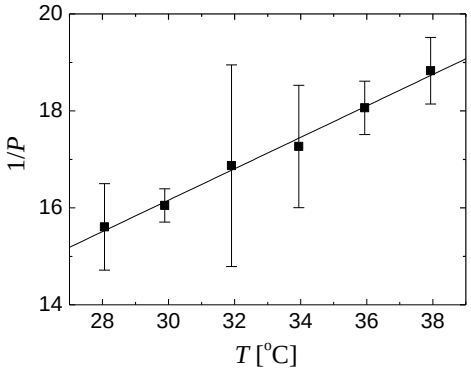
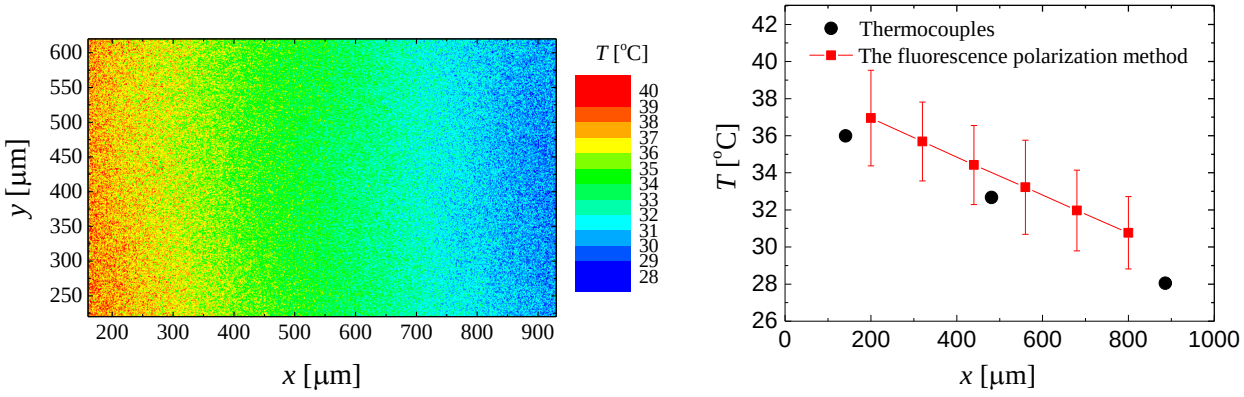


Fig. 4 Relationship between fluid temperature and reciprocal of P of C-FITC solution. The solid line is based on the least mean square approximation.



(a) Two-dimensional distribution (b) Temperature in the x direction

Fig. 5 The measured temperature distribution in microchannel: (a) Two-dimensional distribution, (b) Averaged temperature in the region with the size of 320μm × 100μm dividing the overall area into six segments.

Temperature measurement. Temperature measurement was then performed in the case when a temperature gradient was generated in the microchannel to validate the local temperature measurement. The temperatures of the left and right of the thermocouples among the three ones shown in Fig. 2(b) were controlled to be 36°C and 28°C, respectively. The distance between these thermocouples was 743μm and temperature difference is 8°C. Therefore, linear temperature gradient of -10.6°C /mm was generated in the channel.

Figure 5(a) shows the two-dimensional temperature distribution in microchannel measured by the present method. As the *x* value increases, the measured temperature decreases. The result qualitatively agrees with the temperature gradient generated by the stage. In order to evaluate this temperature gradient quantitatively, temperature in the six regions were averaged. These regions had the area of 320μm × 100μm and were located to be equally spaced in the flow direction (*x* axis). The results are shown in Figure 5(b) by the square symbols. The black circles shown in the graph present the temperature measured by the thermocouples attached to the backside of the channel bottom wall. The measured value using the fluorescence polarization method decreases linearly with *x*. Comparing the results obtained by *P* and thermocouples, both slopes agree well with each other: the temperature gradient of both measurements are -10.3°C /mm and -10.6°C /mm, respectively. Fig. 5(b) also shows that the absolute values measured by the present method were little larger than the thermocouple measurements. There is a possibility that the positions of the thermocouples were slightly shifted to the right. Due to the thermocouple thickness, it was difficult to attach the thermocouples in the accuracy of micro meter scale.

5. CONCLUSIONS

Experiment was performed to study the feasibility of the fluid temperature measurement using the fluorescence polarization method. The fluid viscosity effects on *P* showed a negative correlation and agreed well with theoretical values. Using Casein-FITC conjugated molecule solution, the reciprocal value of *P* showed a linear relationship with the fluid temperature and accuracy of ±2.99°C. Further, the temperature distribution in the microchannel was measured and compared with the values measured by thermocouples. The results agreed well and confirmed the feasibility of the present method.

NOMENCLATURE

B	systematic error	(-)	T	temperature	(K)
d	diameter	(nm)	V	volume	(m^3)
$I_{\parallel}$, $I_{\perp}$	fluorescence intensity	(-)	θ	angle	(rad)
k_B	Boltzman constant	(J/K)	μ	viscosity	(Pa· s)
P, P_0	fluorescence polarization degree	(-)	τ	fluorescent life time	(s)

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