Quantification of fluid temperature field using fluorescent anisotropy

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ABSTRACT

A precise temperature management of liquid in microscale is strongly required for an advanced control of microreactor performance and microfluidic analysis of biomolecules or DNA. In this study, we have developed a measurement technique of temperature field in microscopic domain using fluorescent anisotropy. Irradiating linearly-polarized excitation light to a fluorescent molecule, the molecule emits a partially depolarized fluorescence due to the rotational Brownian motion of the molecule in liquid related to the liquid temperature. An analysis of the polarization degree of the emitted fluorescence, fluorescent anisotropy, results in the liquid temperature mapping using a single fluorophore which does not depend on the illumination intensity variation and dye concentration distribution. In our developed system, both parallel and vertical polarized fluorescent images were obtained by a mechanical switching with image acquisition intervals of 1 s. Preliminary experiments showed that the fluorescent anisotropy has negative temperature coefficient. In order to optimize the temperature dependence of the fluorescent anisotropy, viscosity dependence on the temperature coefficient of fluorescent anisotropy was evaluated. From this evaluation, it was found that temperature dependence of fluorescent anisotropy drastically changed in low viscosity liquid and does not change in highly viscous one larger than 50 mPa·s. In addition, as a verification of the developed system and method, fluorescent anisotropy of liquid in a depth-varying microchannel was measured. The fluorescent anisotropy showed uniform value even though the fluorescent intensity was drastically changed due to the difference in the channel height. In this measurement, standard deviation of the anisotropy image of 0.0015 was achieved; corresponding to the temperature fluctuation of 0.35 K. Furthermore, the temperature distribution of liquid under a local laser heating was obtained. Spatial resolution estimated from the Rayleigh criterion was approximately 680 nm. From these results, the effectiveness of the anisotropy-based thermometry for a microfluidic platform was indicated.

1. Introduction

Recently, a microreactor as a small chemical reaction system with microchannel network has been attracting attention (Mason et al. 2007). Due to the scale effect in heat transfer, enhanced heat exchange efficiency resulting in rapid heating and cooling is achieved in the microreactor-based reaction platform (Kakuta et al. 2001). In order to take advantage of this feature, control of the temperature around the reaction field is essential. In other examples, temperature
measurement in microfluidic device is also important in assessing performance of polymerase chain reaction (PCR) which is a technique to amplify a piece of DNA. Since the PCR relies on thermal cycle consisting of repeated heating and cooling, the control of the temperature in each step is important (Giordano et al. 2001). There have been various methods to measure the liquid temperature in microfluidic systems, such as micro thermocouple (Watanabe et al. 2005) and the laser-induced fluorescence (LIF) (Ross et al. 2001; Motosuke et al. 2009). Particularly, LIF can be a powerful tool to measure the liquid temperature because of its nature of invasive manner, however, LIF is not suitable for a temperature evaluation in a cellular or a microchannel with complicated geometry (Baffou et al. 2009). Two-color LIF which utilizes another fluorophore without temperature sensitivity in fluorescent intensity can be applied in the microchannel with complicated geometry (Natrajan et al. 2009). However, two-color LIF can be used under an assumption of uniform concentration of two fluorescent dyes in the fluidic system. In this sense, multiple fluorophore-based thermometry has the disadvantage.

In this study, we focused on fluorescent anisotropy. Fluorescent anisotropy has tolerances to non-uniformity in molecule concentration or incident light intensity and to fluorescent quenching (Donner et al. 2012). Thus, fluorescent anisotropy based thermometry has a potential to be applied for temperature field imaging not only in the microscopic but also macroscopic domain. In this study, we propose a microfluidic temperature field imaging method using fluorescent anisotropy which has independences of fluorescent quenching and observation depth.

2. Principle of temperature measurement based on fluorescent anisotropy

2.1 Fluorescent anisotropy

When a linearly-polarized excitation light is irradiated to a fluorescent molecule in a medium, fluorescence which is emitted from the molecule is depolarized due to the rotational Brownian motion of the molecule related to the liquid temperature (Fig. 1). Fluorescent anisotropy, \( r \), is a value of the polarization degree of the emitted fluorescence normalized by the total fluorescent intensity and is defined as Eq. (1) (Lakowicz 2006).

\[
r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}}
\]  
(1)
Fig. 1 Schematic of fluorescent anisotropy. Fluorescence dye which has parallel absorption moment to incident light is selectively excited.

Where $I_{||}$ and $I_{\perp}$ are the parallel and vertical component of fluorescent intensity to the polarization direction of excitation light, respectively. The fluorescent anisotropy relates to the temperature of the liquid.

### 2.2 Temperature measurement principle using fluorescent anisotropy

Here, the relationship of the fluorescent anisotropy and the fluid temperature is simply explained. According to Perrin’s equation, the measured value of fluorescent anisotropy is presented as Eq. (2) (Lakowicz 2006).

$$r = \frac{1}{r_0} \left( 1 + \frac{\tau_F}{\tau_R} \right)$$  \hspace{1cm} (2)

Where $r_0$, $\tau_F$ and $\tau_R$ are the limiting anisotropy, fluorescence lifetime and rotational correlation time, respectively. The limiting anisotropy is the theoretical polarization anisotropy $r_0$ without any molecular motion (Johansson 1990). The rotational Brownian motion of fluorescent molecule and excitation energy transfer between fluorescent molecules are the factor that varies the fluorescent anisotropy. Among the parameters given above, the rotational Brownian motion has a temperature dependence. The more increasing the molecule rotation becomes during fluorescent lifetime, the less information about polarization of incident light is obtained. In other words, the value of fluorescent anisotropy decreased as the decrease of the polarization angle of fluorescence by rising temperature. Then, the rotational correlation time is presented as Eq. (3) according to Stokes-Einstein equation (Lakowicz 2006).
\[ \tau_R = \frac{V\eta(T)}{k_BT} \]  

Where \( T \) the temperature of the fluid, \( \eta(T) \) the fluid viscosity, \( V \) the hydrodynamic molecular volume and \( k_B \) the Boltzmann constant, respectively. Because the anisotropy is normalized by its total light intensity, there is no necessity to use any other fluorescent dye without temperature sensitivity to obtain the temperature field independent of incident irradiance uniformity or number of molecules in the measurement volume which would occur in the liquid filled in a channel with complicated geometry.

3. Experimental system

To obtain the fluorescent anisotropy, an experimental system shown in Fig. 2 was developed. A mercury lamp was used as the excitation light. The light is linearly polarized by a polarizer (P), and illuminates a microfluidic device. The microfluidic device was made from PDMS (polydimethylsiloxane) microchannel with a height of 40 \( \mu \)m and width of 2 mm. Fluorescent images both parallel and vertical polarization were obtained by a mechanical switching device (Ps) with mutually orthogonal polarizers with image acquisition intervals of 1 s. A diode laser with a wavelength of 640 nm was used as a source for local heating. A sample solution consists of fluorescein (0.1 mM), glycerol ammonia water solution and brilliant blue FCF (1 mM) as temperature-sensitive fluorescent dye, medium and absorbing dye for the heating laser, respectively.

4. Measurement results

4.1 Viscosity dependence of temperature dependence

Temperature dependence of fluorescent anisotropy becomes maximum when the rotational correlation time \( \tau_R \) is equivalent of the order of the fluorescence lifetime \( \tau_F \) as indicated in Eq. (2). Hence, from relationship between Eqs. (2) and (3), the temperature dependence can be enhanced by adjusting the liquid viscosity. The temperature dependences of the fluorescent anisotropy of fluorescein under different viscosities were measured using a spectrofluorometer. The temperature dependence at each viscosity is depicted in Fig. 3. From this result, it is confirmed that the fluorescent anisotropy has high temperature coefficient under highly viscous solution.
Fig. 2 Schematic of temperature measurement system based on fluorescent anisotropy. An incident light is polarized by a polarizer (P). Parallel and vertical component of fluorescent intensity is obtained by a switching device (Ps).

To obtain the high temperature dependence on the fluorescent anisotropy, glycerol : ammonia water solution (= 4 : 1 in weight) with a viscosity of about 50 mPa·s was used as a solvent.

4.2 Temperature coefficient of fluorescent anisotropy

Since the fluorescent anisotropy is a value that varies depending on the temperature, it is necessary to obtain a calibration curve as a temperature coefficient in advance. Fig. 4 shows the temperature coefficient of fluorescent anisotropy from room temperature (25 °C) to approximately 60 °C. The temperature gradient of fluorescent anisotropy of fluorescein was approximately -0.0045 K⁻¹. The temperature measurement of the fluid can be achieved at the calibration range using this temperature coefficient.
Fig. 3 Relationship between solution viscosity and temperature coefficient of fluorescent anisotropy. The temperature coefficient has strong dependence in low viscosity and does not change over about 50 mPa s.

Fig. 4 Relationship between fluorescent anisotropy and temperature. Fluorescent anisotropy of fluorescein has negative temperature dependence of about -0.0045 K⁻¹.

4.3 Depth dependence of fluorescent anisotropy

To confirm the validity of the anisotropy thermometry as reliable microfluidic temperature imaging technique which has independence of the variation of fluorescent intensity, a channel with a step having different channel height in a measurement area was used. As shown in Fig. 5(a), it was confirmed that the fluorescent intensity varies before and after of the step. On the other hands, the anisotropy yielded uniform distribution as shown in Fig. 5(b). From Fig. 5(b), standard deviation of the fluorescent anisotropy was 0.0015, which corresponds to the
temperature fluctuation of 0.35 K. As a result, it was indicated that the anisotropy-based temperature measurement is of independence of fluorescent intensity.

4.4 Measurement of a temperature field under local laser heating

Moreover, as a verification of the effectiveness of the developed system, a temperature field of the solution under a local laser heating was conducted. The optical power of the diode laser to be irradiated ranged from 0 to 5 mW. The results in Fig. 6 indicate that the temperature in the center area of the laser irradiation increases with the optical intensity.

5. Conclusions

A microfluidic temperature imaging method using a fluorescent anisotropy was established. Relationship between temperature dependence of fluorescent anisotropy and liquid viscosity was evaluated. Then the temperature coefficient of the fluorescent anisotropy in the range about 25 to 60 °C was obtained. Also, the fluorescent anisotropy of the liquid in a microchannel with a step and the temperature field under a local laser heating were measured. These results imply that the independence of depth direction of fluorescent anisotropy and the validity of an anisotropy-based thermometry for a microfluidic platform.
Temperature rise of the solution under a local laser heating field at each optical intensity of a diode laser.

Temperature rises depend on the laser power.

References


