TFEC9-1420

FLUID VISCOSITY MEASUREMENT IN MICROCHANNELS USING FLUORESCENCE POLARIZATION IMAGING

R. Kuriyama*, T. Nakagawa, K. Tatsumi and K. Nakabe

Department of Mechanical Engineering and Science, Kyoto University,
Kyotodaigaku-katsura, Nishikyo-ku, Kyoto 615-8540, Japan

ABSTRACT

This study describes the development of a non-contact and two-dimensional fluid viscosity measurement technique using fluorescence polarization microscopy. When fluorescent molecules dissolved in liquid are excited by a linearly-polarized light, they experience rotational Brownian motion during the period from excitation to emission. Since fluorescence depolarization is observed with the degree influenced by the extent of their rotation which is related to the fluid temperature, viscosity, and molecular size, fluid viscosity can be determined under a fixed temperature condition by measuring steady-state fluorescence polarization degree (P). Concerning the viscosity dependences of P, three different fluorescent molecules were compared in a preliminary experiment and casein molecule labeled with fluorescein isothiocyanate (casein-FITC) was chosen among them as a suitable probe. A calibration experiment was then carried out using casein-FITC in a straight microchannel at a constant temperature of 30 °C. The calibration result showed a relationship that the reciprocal of P linearly increased with the reciprocal of viscosity, which qualitatively agreed with the theoretical prediction. By applying this calibration result, a viscosity gradient was clearly visualized in a microchannel in which co-flowing two solutions of different viscosities interdiffused. These results indicate the potential of the present technique for contributing to microscale analyses of chemical and biological fluids.

KEYWORDS: Fluorescence polarization, Fluid viscosity measurement, Microchannel, Casein-FITC

1. INTRODUCTION

Determination of local viscosity in microscopic scale is of key importance especially for biological samples, such as blood plasma[1] and living cells[2] since it is directly linked with composition change and mass transport of biomolecules. It can provide useful information to understand various reactions and kinetics of biological processes; blood clotting and cell signaling, for instance. Local viscosity measurement also offers an aid to develop diagnostic and therapeutic approaches as the viscosity can be an indicator of some diseases[1].

However, the wide-spread mechanical viscometers, such as capillary viscometer and rotational viscometer, measure bulk viscosity of liquid, i.e., viscosity averaged over the whole sample. To obtain the microscopic viscosity of samples in low volume, various measurement techniques based on optical sensing have been proposed in recent years, including fluorescence imaging of molecular rotors[2], Brownian microscopy [3], steady-state or time-resolved fluorescence polarization[2,4,5], fluorescence lifetime imaging[5] and so forth.

Among these techniques, the present study employs steady-state fluorescence polarization which exploits depolarization due to rational Brownian motion of fluorophores. The main advantage of the technique is that planar distributions of fluid viscosity can be visualized with relatively simple experimental arrangement compared to time-resolved measurements. Moreover, unlike the techniques using molecular rotors, any fluorescent molecule can be used as a probe if the molecular size and fluorescence lifetime are suitable for the intended viscosity range. In the present study, we focus on a development of a visualization method for

*Corresponding Author: kuriyama@me.kyoto-u.ac.jp

viscosity distributions in microscale based on fluorescence polarization and demonstrate a two-dimensional measurement in microchannel flow to validate the feasibility of this method.

2. MEASUREMENT PRINCIPLE

When randomly oriented fluorophores are irradiated by a linearly-polarized excitation light, only those with the absorption moments appropriately aligned to the excitation polarization direction can absorb the light. Specifically, the probability of the absorption is proportional to the value $\cos^2\theta$, where θ is the angle between the directions of excitation polarization and absorption moment. If the excited fluorophores are at stationary condition, they emit the fluorescence polarized in the same direction with the absorption moment (Fig. 1(a)). On the other hand, if the excited fluorophores are suspended in fluid and thus free to rotate, they experience rotational Brownian motion during the period from excitation to emission (Fig. 1(b)). Consequently, the emission moments, which are initially oriented along the absorption moments, are randomized according to the extent of their rotation. Depolarization of fluorescence is therefore observed with the degree which is related to the fluid temperature, viscosity, and the molecular size.

Polarization degree, P, is defined as a function of $I_{\parallel}$ and $I_{\perp}$, which are the fluorescence intensities of the components that are parallel and perpendicular to the polarization direction of the excitation light, respectively:

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

Perrin [6] theoretically derived the equation which relates this P value to the fluorescence lifetime and the rotational diffusion of fluorescent molecules as

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

where P_0 is the intrinsic polarization defined as the polarization degree of the molecule at stationary condition (in absent of rotation), k_B is the Boltzmann constant, V is the hydrodynamic volume of the rotating unit, and τ is the fluorescence lifetime. T and μ are the absolute temperature and viscosity of the fluid, respectively. Clearly seen from Eq. (2), the reciprocal of P shows a linear relation with T/μ. Therefore, if V and τ remains constant during the measurement, the fluid viscosity, μ, can be determined by measuring P under fixed temperature condition.

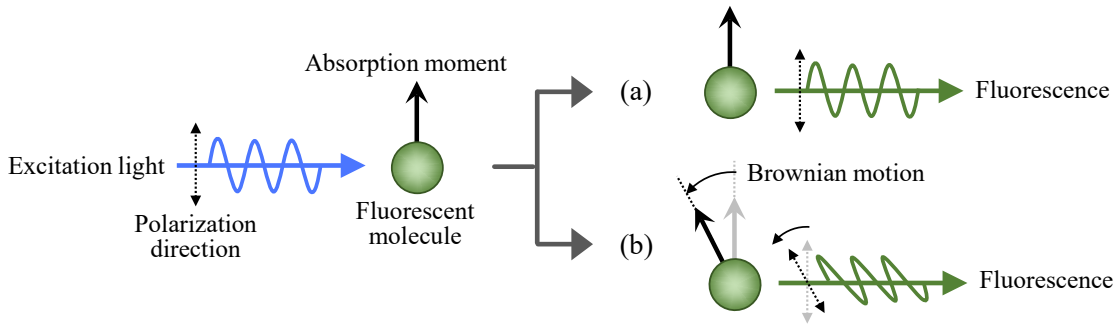

Fig. 1 Polarization direction of the fluorescence emitted from (a) a molecule in stationary condition and (b) that suspended in fluid.

3. EXPERIMENTAL SECTION

3.1 Measurement System Figure 2(a) shows a schematic of the optical measurement system using an upright microscope (Olympus, BX51). Polarization measurements can be performed by either transmission or epi-fluorescence observation. A light from LED was linearly-polarized by a polarized light condenser or a polarization plate, filtered by an excitation filter and focused to the sample on the microscope stage. The fluorescence collected

by an objective lens (Olympus, LMPlanFLN10x) was filtered by a fluorescence filter and captured by a photomultiplier (Hamamatsu Photonics, H5783-03) or a sCOMS camera (Andor, Zyla 4.2 plus) through a second polarization plate mounted on a rotation stage. Only for epi-fluorescence observation, a dichroic mirror was placed in the optical path. Note that the LED light source and the set of filters were appropriately selected with consideration of the fluorescence characteristics of the fluorophore used as a probe in the polarization measurement. The temperature of the microscope stage was controlled by circulating water from an isothermal bath so that the temperature of sample solution was maintained at 30 °C.

3.2 Microchannel Sample solution was enclosed in a prepared slide (consisting of bottom and upper cover glasses spaced by two strips of 120-μm thick parafilm), or introduced into a microchannel at a constant flow rate using a syringe pump. Figure 2(b) shows a schematic of a Y-shaped microchannel used for two-dimensional viscosity measurement. The microchannel was comprised of a PDMS chip and a cover glass, which were bonded by oxygen-plasma treatment. K-type thermocouples were inserted in 1-mm diameter holes made by biopsy punch and the channel temperature was logged during the polarization measurement.

3.3 Chemicals The present study employed fluorescein (diameter = 1.0 nm[7], molecular weight = 332, Nacalai Tesque, #16106-82), rhodamine B (dia.= 1.6 nm[8], MW = 479, Kishida Chemical, #000-68232), and casein molecules labeled with fluorescein isothiocyanate (casein-FITC, dia.= 30 nm, MW = 26000, AAT Bioquest, #13440) as fluorescent probes. In anticipation of the future application to biological samples with relatively low viscosity, such as human blood plasma, the viscosity of sample solution was controlled in the range 0.8–2.8 mPa·s by adjusting glycerol concentration to 0–40 wt%.

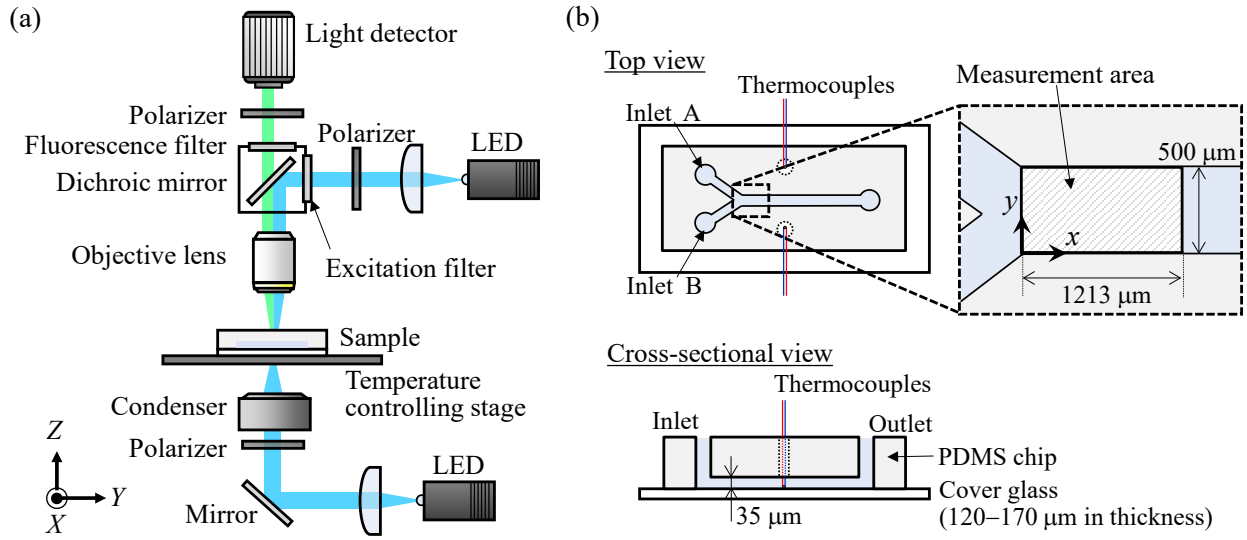

Fig. 2 (a) Schematic of the optical system for fluorescence polarization measurement and (b) top and cross-sectional views of a Y-shaped microchannel used for two-dimensional viscosity measurement.

4. RESULTS AND DISCUSSIONS

4.1 Selection of Fluorescent Probe In Eq. (2), $1/P$ theoretically shows linear increase with $1/\mu$ and the slope changes with τ and V. Therefore, the sensitivity and precision of the present technique highly depend on the characteristic properties of the fluorescent probe. In order to select an appropriate probe, polarization measurements were carried out using fluorescein, rhodamine B, and casein-FITC. A prepared slide filled with sample solution was set on the microscope stage and fluorescence intensities, $I_{\parallel}$ and $I_{\perp}$, were successively measured with transmission observation. The fluorescence intensity was detected by the photomultiplier at 5 Hz and accumulated for 2 s. Figure 3(a) shows the measurement result. $1/P$ increases with $1/\mu$ in all cases, however, linear correlation is not obtained in fluorescein case. In rhodamine B and casein-FITC cases, $1/P$ shows proportional increase with $1/\mu$ and the slopes are nearly the same: measurement sensitivities for viscosity

measurement are equivalent in the two cases. On the other hand, measurement precision is expected to be higher in casein-FITC case since the absolute value of P is larger compared with rhodamine B. Casein-FITC was selected as a probe in the present study according to these discussions.

4.2 Calibration Experiment Prior to a two-dimensional viscosity measurement, a calibration experiment was conducted using 0.1 wt% casein-FITC solutions in a straight microchannel with height of 35 μm and width of 500 μm. The epi-fluorescence configuration and the sCOMS camera were used for the experiment. The exposure time was set at 1s and 10 fluorescent images were captured for each intensity component. P was then calculated by averaging 10 polarization distributions temporally and spatially. Figure 3(b) shows the calibration result, where each plot indicates the averaged value of four measurement data and the error bar shows the uncertainty at 95% confidence level. It is clearly seen that $1/P$ linearly increases with $1/\mu$, which agrees well with the theoretical prediction shown in Eq. (2). The dashed line shown in Fig. 3(b) was derived by linear least-square regression and used as a calibration line for viscosity measurement in the following section.

4.3 Viscosity Distribution Measurement Based on the calibration result, two-dimensional viscosity measurement was performed in the Y-shaped microchannel (Fig. 2(b)). 40 wt% glycerol solution (2.8 mPa·s) and pure water (0.8 mPa·s) were injected from inlets A and B, respectively, at the same flow rate of 0.28 μL/min. Polarization measurement was carried out with the same optical configuration and exposure time as the calibration experiment. Figure 4(a) shows the two-dimensional viscosity distribution visualized at a spatial resolution of 1.4 × 1.4 μm^2 by averaging over 2 × 2 pixels^2. The measured viscosity was about ~2.7 mPa·s in the upper side of the distribution and was ~0.7 mPa·s in the lower side. These results roughly match the viscosities of the two fluids introduced from the corresponding inlets. Moreover, the viscosity gradually changed with y position near the interface of the two fluids by interdiffusion. To obtain further insight into the measurement result, the spanwise distributions at x = 144.4 μm and 1210.1μm were plotted (solid lines) and compared with the result of numerical calculation (dashed lines) in Fig. 4(b). The vertical axis indicates the value normalized between 0–1 by the viscosity averaged over y = 20–120 μm and that over y = 400–500 μm. In numerical calculation, two-dimensional diffusion equation was solved using the diffusion coefficient of glycerol solution (4.0×10^{-10} m^2/s at 40 wt%[9]). Since the actual flow rate was temporally varied due to the pulsation flow of the syringe pump, the position of the interface between two fluids at x = 0 μm was given based on the experimental result. The calculated concentration distribution was converted into a viscosity distribution based on the relationship between glycerol concentration and viscosity which was obtained in preliminary experiment. From the measurement result, the viscosity gradient around 130 μm ≤ y ≤ 230 μm is more moderate at x = 1210.1 μm than x = 144.4 μm, which clearly indicates that the mass diffusion in the spanwise direction progressed along the fluid flow. These results agreed well with the numerical calculation and confirmed the feasibility of the present method.

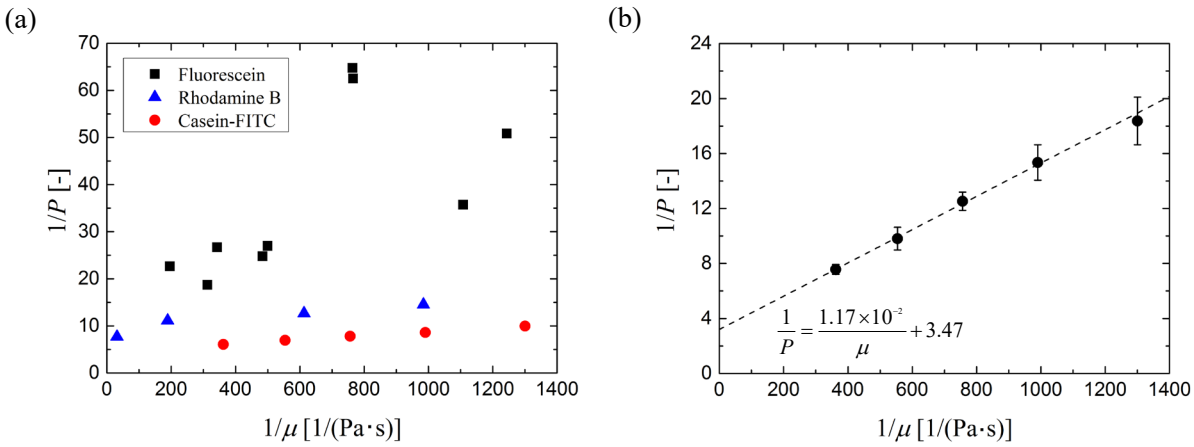

Fig. 3 (a) Comparison of the relationships between $1/\mu$ and $1/P$ obtained for three different fluorescent molecules (fluorescein, rhodamine B, and casein-FITC). Measurements were conducted by transmission observation. (b) Calibration result obtained using casein-FITC by epi-fluorescent observation. The calibration straight (dashed line) was used for two-dimensional viscosity measurement.

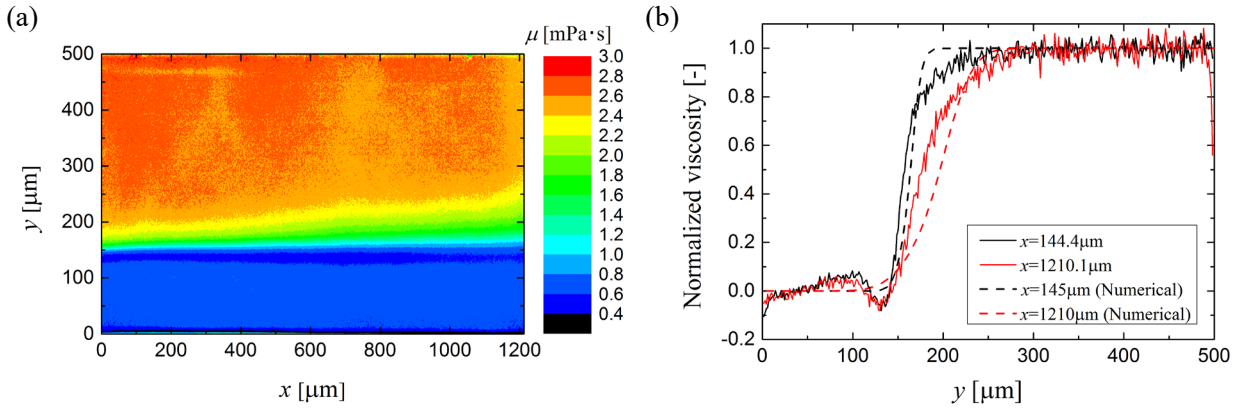

Fig. 4 (a) Viscosity distribution in the Y-shaped microchannel measured by fluorescence polarization. (b) Comparison of spanwise distributions of normalized viscosity at x = 144.4 μm and 1210.1 μm between fluorescence polarization experiment and numerical calculation.

5. CONCLUSIONS

The present study describes the development of a non-contact and two-dimensional fluid viscosity measurement technique using fluorescence polarization and its application to the microchannel flow. In the selection of a fluorescent probe, a good correlation between the fluid viscosity and the polarization degree (P) and the largest P value were obtained in casein-FITC case. A calibration experiment using casein-FITC showed that the reciprocal of P linearly increased with the reciprocal of viscosity in the range 0.8–2.8 mPa·s, which agreed with the theoretical prediction. By applying this calibration result, a viscosity gradient formed by the interdiffusion between co-flowing two fluids was clearly visualized in a microchannel at a spatial resolution of 1.4 × 1.4 μm^2 and agreed well with the numerical calculation. This result confirms the feasibility of the present technique and implies the future application to microscale analyses in chemical and biological fluids.

NOMENCLATURE

$I_{\parallel}$, $I_{\perp}$	fluorescence intensities with parallel and perpendicular polarizations to the direction of excitation polarization (W/m^2)	V	hydrodynamic volume of rotating unit (m^3)
		θ	angle between the direction of excitation polarization and absorption moment (°)
P	polarization degree (-)	μ	fluid viscosity (Pa·s)
P_0	intrinsic polarization (-)	τ	fluorescence lifetime (s)

REFERENCES

[1] Harkness, J. and Whittington, R. B., "The viscosity of human blood plasma: its change in disease and on the exhibition of drugs," *Rheol. Acta* **10**, 55-60 (1971).

[2] Kuimova, M. K., "Mapping viscosity in cells using molecular rotors," *Phys. Chem. Chem. Phys.* **14**, 12671-12686 (2012).

[3] Kim, J. and Ju, Y. S., "Brownian microscopy for simultaneous in situ measurement of the viscosity and velocity fields in steady laminar microchannel flows," *J. Microelectromechanical Systems* **17**, 1135-1143 (2008).

[4] Dix, J. A. and Verkman, A. S., "Mapping of fluorescence anisotropy in living cells by ration imaging," *Biophys. J.* **57**, 231-240 (1990).

[5] Benninger, R. K. P., Hofmann, O., McGinty, J., Isidro, J. R., Munro, I., Neil, M. A. A., deMello, A. J. and French, P. M. W., "Time-resolved fluorescence imaging of solvent interactions in microfluidic devices," *Optic Express* **13**, 6275-6285 (2005).

[6] Perrin, F., "Brownian movement of ellipsoids: 1. Dielectric dispersion for ellipsoidal molecules," *J. Phys. Radium* **10**, 497-511 (1934).

[7] Mustafa, M. B., Tipton, D. L., Barkley, M. D. and Russo, P. S., "Dye diffusion in isotropic and liquid crystalline aqueous (hydroxypropyl) cellulose," *Macromolecules* **26**, 370-378 (1993).

[8] Tsunomori, F., and Ushiki, H., "Pore size effect on diffusion coefficient of rhodamine B in PNIPA gel," *Physics Letters A* **258**, 171-176 (1999).

[9] Nisijima, Y. and Oster, G., "Diffusion in glycerol-water mixture," *Bulletin of the Chemical Society of Japan* 1649-1651 (1960).