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Development of Viscosity Measurement Technique in Microchannel Using Fluorescence Polarization Method

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1. Introduction

Viscosity measurement in microfluidics and biology is an important issue in terms of designing lab-on-a-chip devices and measuring the rheological characteristics and biological and chemical components of the fluid. For example, viscosity of blood plasma changes with the change in its constituents and acts as an index of biochemical reactions and the severity of diseases[1]. Therefore, viscosity measurement in microscale is expected to contribute to elucidation of the pathology and diagnosis with low sample volume requirements. Since such samples easily solidify, it is desirable to measure the viscosity non-intrusively.

In this study, we propose a technique for microscale fluid viscosity measurement based on polarization characteristics of fluorescence which is related to the rotational Brownian motion of fluorescent molecules. Using this method, we can conduct a non-intrusive measurement for the two-dimensional distribution of viscosity with high spatial resolution. This paper describes the measurement technique and the results of measurement for the mass diffusion between parallel flows of two solutions in the microchannel.

2. Methods

2.1 Measurement principle

When a fluorescent molecule dissolved in a solution is irradiated by a linearly-polarized excitation light, the molecule is excited if the direction of its absorption moment is close to the polarization direction of the excitation light. The excited fluorescent molecule emits fluorescence whose polarization direction is parallel to its absorption moment. The polarization degree, P, an index of the fluorescence polarization characteristics, is defined as,

$$P = (I_{\parallel} - I_{\perp})/(I_{\parallel} + I_{\perp}) \tag{1}$$

where $I_{\parallel}$ and $I_{\perp}$ represent the fluorescent intensities of the components parallel and vertical to the polarization of excitation light, respectively. If the viscosity of the solution is low, rotational Brownian motion of the fluorescent molecules gets intense and the directions of their absorption moments are largely randomized by the time they emit fluorescence. As a result, the fluorescence is depolarized and P becomes small. Perrin and Weber[2] theoretically derived the relationship between P and the viscosity, μ, expressed as,

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

where P_0 is the polarization degree of the molecule at stationary state, k_B is Boltzmann constant, T is the solution temperature, τ is fluorescence lifetime, and V is the volume of the fluorescent molecule. Since $1/P$ is proportional to $T\tau/\mu V$, the solution viscosity can be obtained by measuring P if T, τ and V are constant.

2.2 Experimental apparatus

Figure 1 shows a schematic of the optical system for fluorescence polarization measurement. An inverted microscope (Olympus; IX-73), an LED light source (Thorlabs; M490L4), and two high sensitivity cameras (Andor Zyla; 4.2 PLUS, 2048 × 2048 pixels[2], 16bit) were used. The light from the LED was linearly polarized by a polarizer, passed through a bandpass filter (Semrock; FF01-482/35-25) and was loosely focused to the sample on the microscope stage. Sample solution was introduced into a microchannel, which was composed of PDMS and a cover glass as shown in Fig. 2(a). Casein-FITC conjugated (AAT Bioquest; molecular weight: 26000, τ: 4ns, excitation wavelength: 494nm, emission wavelength: 518nm) was dissolved in sample solution as a fluorescent probe at concentration of 4.0×10^{-5}mol/L according to the previous study[3]. The emitted fluorescence was collected by an objective lens (Olympus; LMPlanFL N, 10x), filtered through another bandpass filter (Semrock; FF01-536/40-25) and split into two components ($I_{\parallel}$ and $I_{\perp}$) by a polarizing prism. $I_{\parallel}$ and $I_{\perp}$ were simultaneously measured by camera 1 and camera 2, respectively. The exposure time and the frame rate were set at 1s and 0.990fps, respectively, and 10 images were captured by each camera. The temperature of the stage was maintained at 30℃ ± 0.5℃ and monitored by thermocouples.

2.3 Numerical calculation

Three dimensional numerical computation was conducted for the flow and viscosity distribution in the microchannel using COMSOL Multiphysics (Ver. 4.3a: COMSOL Inc.). Mass and momentum conservations equations of the flow and concentration conservation equation for binary diffusion were solved based on finite element method for steady-state laminar flow. The equation was discretized and solved by employing Galerkin method and restarted GMRES (generalized minimum residual) solver. Figure 2(b) shows the computational domain. At the two inlets of the Y-shaped channel, inlet flow with uniform velocity distribution for liquids of water and 35wt% sucrose-water solution were applied. Neumann condition was applied to the channel outlet. No-slip and zero concentration flux boundary conditions were applied to the channel walls for flow and mass concentration, respectively. Rectangular mesh was used in the computation and the minimum and maximum mesh sizes were 7 × 7 × 2.5 μm^3 and 10 × 10 × 2.5 μm^3, respectively. The number of meshes was 264,810. The effect of concentration on the viscosity, density, and diffusion coefficient of the sucrose solution was considered in the computation. Density was calculated from the reference[4] and the diffusion coefficient was interpolated from the mutual diffusion coefficient of sucrose and water[5].

3. Results and Discussion

3.1 Calibration experiment

Calibration experiments between $1/P$ and $1/\mu$ were conducted using seven sucrose solutions with different concentrations. The solution viscosity was estimated from the relationship between the viscosity and the sucrose concentration which was preliminarily obtained using a rheometer (Anton Paar; MCR301). Each solution was sent into a straight microchannel by a syringe pump at constant flow rate of 0.56μL/min and polarization measurement was carried out. Distributions of P were calculated from the ten pairs of fluorescent images and the distributions were averaged temporally and spatially. Figure 3 shows the calibration result. Each plot shows the averaged value of four measurement data and the error bar shows the 95% confidence interval. As predicted in Eq. (2), $1/P$ increased linearly with $1/\mu$. The straight line was obtained by least squares regression and used as a calibration line. The measurement uncertainty was estimated to be 5~10% from the averaged value of the error bars.

3.2 Viscosity distribution measurement

Viscosity distribution measurement was performed in the Y-shaped microchannel using the calibration result. Water (0.8mPa・s) and 35wt% sucrose solution (2.9mPa・s) were injected into each inlet of the channel at flow rate of 0.6μL/min by syringe pumps as shown in Fig. 2(a). The measurement region and the coordinate system are also shown in the enlarged top view in Fig. 2(a).

Figure 4 shows the measurement result, which was obtained by averaging five instantaneous P distributions. The two-dimensional distribution of μ is visualized at spatial resolution of 1.3μm × 1.3μm based on 2×2 pixel binning. In Fig. 4, measurement result in y < 50μm and y > 150μm agree with the viscosities of water and 35wt% sucrose solution, respectively. The viscosity gradient is observed in 50μm < y < 150μm, and the width of the diffusion layer gets wider in the downstream. These results show that the viscosity distribution influenced by the mass diffusion is successfully visualized.

To examine the validity of the present technique, the measurement result in Fig. 4 is compared with that of numerical calculation. Figure 5 shows the spanwise distributions of the measured and calculated viscosities at x =10μm and 900μm. During the measurement, the position of the solution interface temporally fluctuated because of the pulsating flow generated by syringe pumps. To take this phenomena into consideration in the numerical calculation, the actual positions of the solution interface were determined from the measured instantaneous viscosity profiles and were used to estimate the instant flow rates of the solutions. Viscosity distributions were simulated with the estimated flow rates and then time-averaged. From the experimental results in solid lines, the viscosity gradient is formed at the solution interface (50μm < y < 150 μm) and the gradient becomes smaller in the downstream region due to the diffusion of sucrose and water molecules in the spanwise direction. These distributions quantitatively agree with the results of numerical calculation in dashed lines, which confirms the validity of the present technique based on fluorescence polarization method for the viscosity distribution measurement in microscale.

4. Conclusions

A non-contact fluid viscosity measurement technique was developed using fluorescence polarization. The measurement uncertainty was 5~10% based on the calibration result. The viscosity distribution in the diffusion layer between two solutions was successfully visualized at spatial resolution of 1.3μm × 1.3μm. The measured viscosity gradient agreed well with the results of calculation, which confirmed the validity of the proposed method.

5. References

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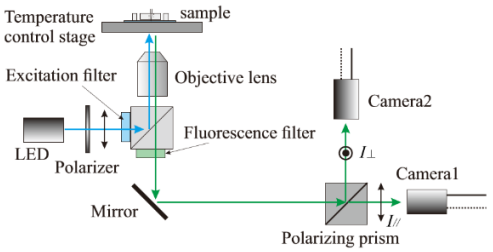

Figure 1. *Optical system for viscosity measurement.*

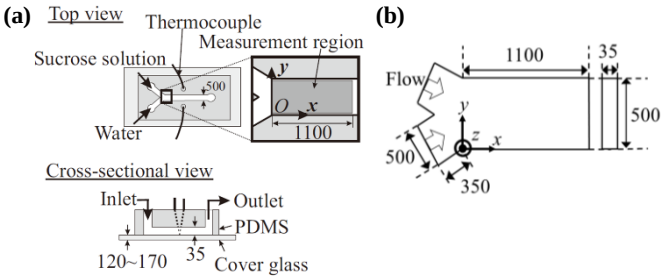

Figure 2. (a) *Top and cross-sectional views of Y-shaped micro-channel.* **(b)** *Computational domain.*

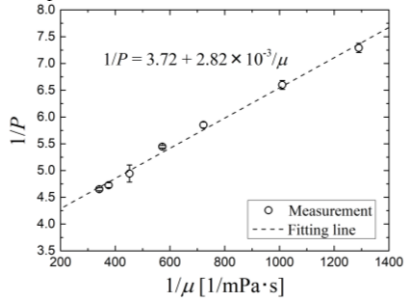

Figure 3. *Relationship between $1/\mu$ and $1/P$.*

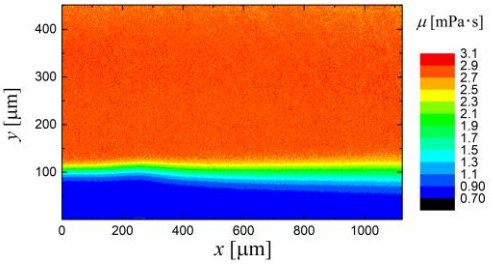

Figure 4. *Two-dimensional distribution of viscosity μ measured in Y-shaped microchannel.*

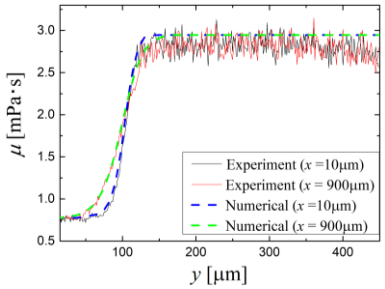

Figure 5. *Spanwise distributions of viscosity μ obtained by fluorescence polarization method and numerical calculation.*