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Fluorescent Dye Probe for Monitoring Local Viscosity of Confined Liquids

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A fluorescence lifetime measurement was performed for monitoring the viscosity of a confined liquid (glycerol) film between mica surfaces using a cyanine dye, Cy1, as a viscosity probe at various film thicknesses. The fluorescence decay curve consisted of two components, i.e., one attributed to the Cy1 in the central part of the liquid between the surfaces and another to the Cy1 adsorbed on the surfaces. The increased lifetime of the former indicated the increase in the viscosity of the confined liquid.

The properties of confined liquids are different from those of the bulk due to the confinement effect and the interaction of liquid molecules with the surfaces. They attract increasing attention because of recent progress in the preparation of many porous materials including nanotubes and nanofabrication processes such as nanoprinting.[1–3] These developments require the full understanding of properties of confined liquids, even a simple one such as viscosity.

Surface force measurements can provide a unique tool for studying confined liquids with varying thicknesses (surface separation, D) at 0.1 nm resolution.[4] Various shear measurement techniques employing the surface force apparatus (SFA) have been developed for characterizing viscosity, lubricity, and stick-slip behaviors.[5–11] A more recent attempt introduced fluorescence correlation spectroscopy to SFA for quantitatively evaluating the diffusion constant of confined liquids.[11] Quantitative determination of diffusion constants is advantageous; however, the optics for conventional distance determination by FECO (fringes of equal chromatic order, using transmitted light) has been replaced by optics for fluorescence, restricting fine regulation of the distance. Further development is necessary for characterizing various properties of confined liquids.

Recently, we have developed a twin-path SFA, which uses laser light reflected on the back of the bottom surface for distance determination.[12] This SFA provides free space at the top of the apparatus for constructing a fluorescence lifetime measurement system under an optical microscope (Figure 1). Using such apparatus, it is possible to measure the fluorescence lifetime of a dye which is sensitive to the viscosity, pH, and polarity of a solvent,[13–16] useful for characterizing confined liquids.

In this study, we used a cyanine dye, 2-[3-(1,3-dihydro-1,3,3-trimethyl-2*H*-indol-2-ylidene)-1-propenyl]-1,3,3-trimethyl-3*H*-indolium iodide, Cy1, as a fluorescent viscosity probe. The viscosity of confined glycerol was monitored by the fluorescence lifetime measurement of Cy1 at various D's, which were controlled using twin-path SFA. It has been reported that nonradiative relaxation of the excitation energy for Cy1 dye occurs through twisting motion around a conjugated hydrocarbon bond.[15] This molecular motion is restricted in viscous solvents, thus we can monitor the viscosity of liquids from the fluorescence lifetime of Cy1.

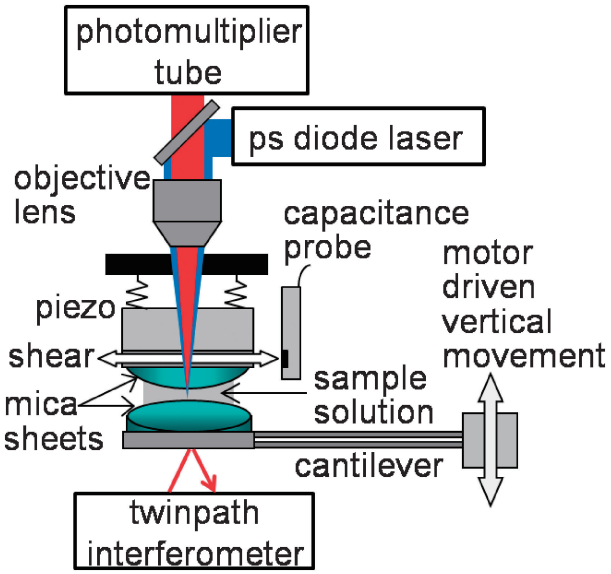

Figure 1. Schematic experimental setups of fluorescence lifetime measurement system within twin-path SFA.

A glycerol solution (20 μM) of Cy1 was placed between mica surfaces mounted in a twin-path SFA placed under an optical microscope (Olympus). The thickness of the liquid film, D, was regulated by the twin-path SFA as previously described.[12] The resolution in the D determination was 1 nm. Pulsed laser light ($\lambda = 467$ nm, 10 MHz, 80 ps, PLP-10-047, Hamamatsu Photonics) was focused through an objective lens (M Plan Apo 5×, Mitsutoyo) on the sample solution between mica surfaces. Emission was collected through the same objective lens and was detected using a photomultiplier tube (Hamamatsu, R7400U-20). The emission signal sent to as ps time analyzer (ORTEC, MODEL9308) through a discriminator (ORTEC, MODEL9327). A fluorescence decay curve was obtained using time correlated single photon counting (TCSPC).[17] The viscosity of bulk solution was measured with a viscometer (Brookfield, RVD-II+ Pro).

First, we determined the dependence of the fluorescence lifetime of Cy1 in bulk solutions on the viscosity of the solutions. The viscosity was varied by mixing high viscosity glycerol (994 cP at 22 °C) and low viscosity ethanol (1.2 cP at 20 °C) at appropriate ratios and also changing the temperature. The decay curves of Cy1 in bulk solutions showed a single-exponential decay. The lifetime of Cy1 in a 1:1 mixture of

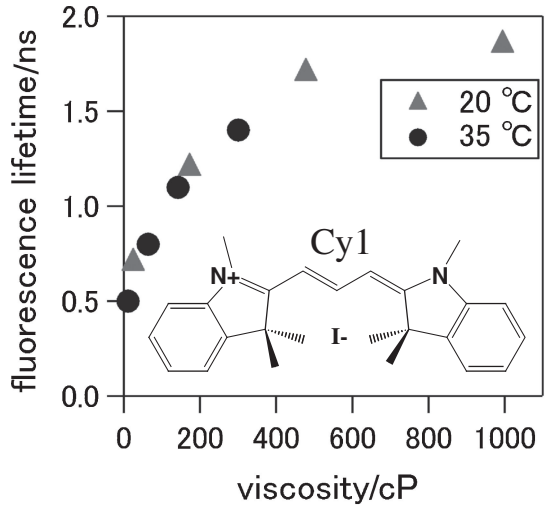

Figure 2. Dependence of fluorescence lifetime of Cy1 on solvent viscosity. Inset: The molecular structure of Cy1.

ethanol and glycerol ($\eta = 11$ cP) was 0.5 ns at 35 °C. The lifetime increased with increasing the viscosity, then reached a plateau (1.7 ns) above 500 cP (Figure 2). Polarity of solvents is known to affect the electronic states of cyanine dyes, thus the fluorescence lifetime of them.[16] We measured UV–visible absorption spectra of Cy1 in neat ethanol and glycerol, of which polarities (dielectric constant) are 25.3 and 46.5, respectively.[18] They showed almost identical spectra indicating that polarity of solvents slightly changed the electronic state of Cy1, and the lifetime changes of Cy1 shown in Figure 2 are not attributed to the polarity of solvents but to viscosity of solvents. Therefore, the lifetime of Cy1 in Figure 2 can be used to determine the viscosity of similar solvents. For the confinement experiments, we choose the glycerol at 35 °C (301 cP) as a sample liquid for conducting the measurement in the sensitive region below 500 cP. We measured fluorescence spectrum of Cy1 in confined glycerol at $D = 10$ nm, which showed identical as that in bulk. This result indicated that no aggregates were formed in confined glycerol and that only fluorescence from Cy1 monomer was observed.

Figures 3a and 3b show the fluorescence decay curve of Cy1 in glycerol at $D = 10$ nm and its residual plot, respectively. The curve was well fitted to a double-exponential function after convoluting it with the instrumental response function. Here, a noise signal of a decay constant of 6 ns, which was from the equipment and was detected even without a Cy1 solution, was also subtracted from raw data of emissive curve (Figure S1 of Supporting Information[22]). The lifetimes of a shorter and a longer component were 0.80 and 1.82 ns, respectively. All decay curves of Cy1 in glycerol for the thickness range from $D =$ 10 nm to 3.8 μm were also fitted by a double-exponential function after subtracting the noise component (less than 1% of the total signal at the maximum at $D = 10$ nm) of the decay constant of 6 ns, which was identical in all measurements. Chi-square value χ_2 is 1.51 in the case of fitting in Figure 3a. This value was slightly larger than the values in the well-fitted case ($\chi_2 < 1.3$–1.5).[17] It indicated that our simple model considering two states of dye molecules in the confined glycerol was acceptable. However, we cannot deny the possibility of some

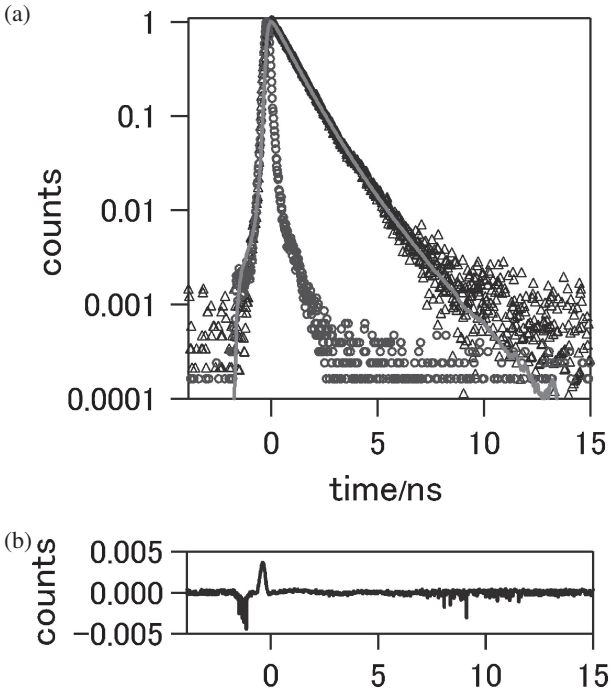

Figure 3. (a) The fluorescence decay curve (△), the instrumental response function (○), and the fitting curve of fluorescence decay (line) for Cy1 glycerol solution confined between mica surfaces at $D = 10$ nm. (b) Residual plot of the fitting curve of the fluorescence decay.

contribution from multiple states corresponding to the viscosity gradient, which might attribute to a slightly larger χ_2.

The two components of emission signals from Cy1, indicating that the Cy1 molecules in the confined solutions were in two different states. Figure 4a plots their lifetimes as a function of D. The longer lifetime of component A (τ_A) was sharply increased from 1.62 ± 0.02 to 1.82 ± 0.02 ns with decreasing D from 30 to 10 nm. On the other hand, the lifetime of the shorter component B (τ_B) was 0.78 ± 0.03 ns and independent of D. The percentage of component A (τ_A) in the total Cy1 fluorescence intensity decreased, while that of component B (τ_B) increased with decreasing D. They reached 27% and 73% at $D = 10$ nm, respectively (Figure 4b). These changes in τ_A and τ_B and the intensities suggested that the Cy1 molecules of component A were dissolved in the center of the confined liquid between the mica surfaces and that those of component B were located in the vicinity of the mica surfaces. We reported that dye molecules were concentrated to more than several 100 mM when they were in liquids confined between two mica surfaces.[19,20] This is the reason that the fluorescence intensity ratio of component A did not increase proportionally with increasing thickness of the liquid. τ_B is shorter than the lifetime for the bulk solution, which should be explained by the concentration quenching of concentrated cationic Cy1 adsorbed on mica surfaces.

It is possible to estimate the viscosity of confined glycerol based on the lifetime of τ_A. The viscosity estimated from τ_A using the calibration curve (Figure 2) was plotted as a function of D in Figure 4c. The viscosity at 3.8 μm was 320 cP and slowly increased to $D = 30$ nm (450 cP), then more sharply

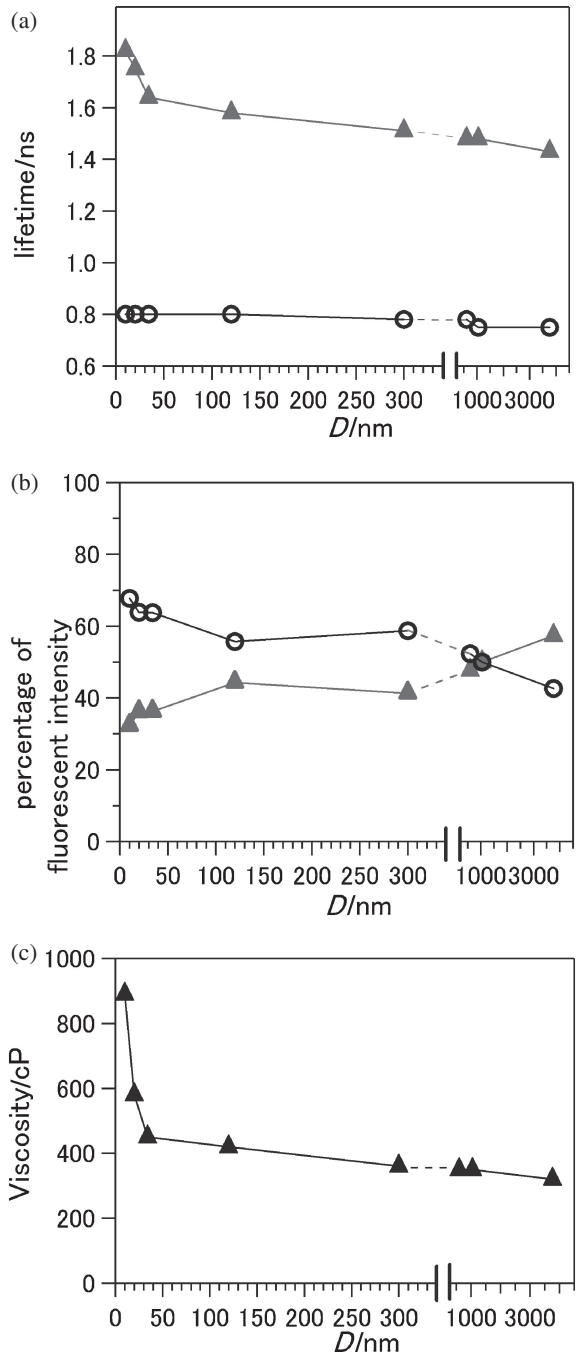

Figure 4. (a) Fluorescence lifetimes of component A (τ_A, ▲) and B (τ_B, ○) for emission of Cy1 in confined glycerol at various D's. (b) Percentage of fluorescent intensity (α_A, ▲ and α_B, ○) at various D's. (c) Estimated viscosity from τ_A at various D's.

below 30 nm to 890 cP at 10 nm. These viscosity changes agreed well with the viscosity determined by the resonance shear measurement we also recently developed.[9,10] It is reported that viscosity of liquids increased under confinement because liquid structuring induced by restriction of molecular motion and the interaction of the molecules with the surfaces.[6,8–10,21] In the case of simple molecules such as water and octamethylcyclotetrasiloxane, liquid structuring occurs in the range of the liquid thickness below 10 nm.[10,21] Structuring of glycerol was observed when we decreased the thickness below 30 nm which is larger than the thickness in general cases. Hydrogen-bonding interactions between three hydroxy groups of glycerol should be responsible for structuring of liquids of such larger thickness.

An instrument which can monitor the fluorescence lifetime of confined liquids with varying film thickness has been developed. We evaluated the viscosity of confined glycerol from the lifetime of Cy1. It slowly increased when D decreased from 3.8 μm (320 cP) to 30 nm (450 cP) and sharply increased from 30 to 10 nm (890 cP). With a long fluorescence lifetime and known relaxation paths, Cy1 was proven a useful probe for monitoring the viscosity of confined liquids of any geometry including tubes and pores because fluorescence lifetime measurement is simple. The fluorescence lifetime measurement system which we developed in this study can be applied to characterization of other local properties as well as photoreactions which are in progress in our laboratory.

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22 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

