형광상관분광법을 이용한 단일 분자의 확산 현상 연구

Single-molecule Translational Diffusion Dynamics Investigated by Fluorescence Correlation Spectroscopy

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Fluorescence correlation spectroscopy (FCS) is a widely used technique in biology, chemistry, and physics [1,2]. By analyzing temporal fluctuation of fluorescence intensity from dye molecules freely diffusing in and out of a small excitation volume in confocal microscopy, the origins of fluorescence fluctuation, such as translational and rotational diffusion and photophysics of dye molecules can be investigated in single-molecule level. As the diffusion dynamics of dye molecules is under the influence of the properties of solution in which the dyes are dissolved, FCS is a suitable tool to characterize liquids and other fluids [3,4]. Here, we study translational diffusion dynamics of dye molecules in glycerol-water mixtures.

We build FCS setup based on a homebuilt confocal microscope. A 532 nm CW laser (CrystaLaser, TEM00 Mode) beam is used as a excitation source and is focused in the middle of a sample by a high numerical aperture objective lens (NA 1.2, water immersion, Olympus). Fluorescence is collected by the same objective lens and spatially filtered by a confocal pinhole (62.5 μm radius) to eliminate the background from out-of-focus region. The confocal detection volume is about 1 femtoliter and is small enough to have only a few molecules in it with the concentration of dye at ~1 nM. A dichroic mirror is used to separate the fluorescence from excitation beam, and a band pass filter (Semrock, F01-585-40) blocks elastic and inelastic scattering from solvent. An avalanche photodiode (Perkin Elmer, SPCM-AQ4C) is used to detect fluorescence signal, and is recorded by a counting board. Correlation function, $G(\tau)=\langle F(t)F(t+\tau)\rangle$, is calculated by home-built software written in LabView, where $F(t)$ is measured fluorescence signal and τ is a delay time. All experiments are performed at room temperature.

The viscosity of the glycerol-water mixture changes dramatically as the glycerol/water ratio changes; the viscosity of pure glycerol is almost thousand times higher than that of water. As the diffusion of dye molecules is governed by viscosity of the solution according to the Stokes-Einstein relation, we performed FCS experiment to demonstrate the viscosity effect on translational diffusion dynamics of tetramethyl rhodamine dye (TAMRA, Invitrogen). Figure 1-(b) shows normalized correlation curves of tetramethyl rhodamine in the mixture with different glycerol-water ratio. For the pure water case (circles in Fig. 1-b), the correlation function decays in sub-millisecond range. The characteristic time at which the normalized correlation function has a value 0.5 is called

diffusion time, and corresponds to average residence time of dye molecules in the confocal detection volume. As the glycerol content increases, the diffusion time of the dye increases up to tens of millisecond indicating slowing down of diffusional motion of dye molecules in viscous solution. The second decay faster than the diffusion dynamics is due to the photophysics of dyes and excluded out during the analysis.

In summary, we demonstrate FCS using confocal microscopy and study diffusion dynamics of tetramethyl rhodamine in glycerol-water mixture. The diffusion of the dye molecules slows down as expected as the viscosity of the mixture increases. Our result shows that FCS is a good tool to investigate the properties of liquids and other fluids. As FCS detects only a small region of a sample, it is suitable to study systems having special inhomogeneity thus making this technique relevant in many chemical and biological investigations.

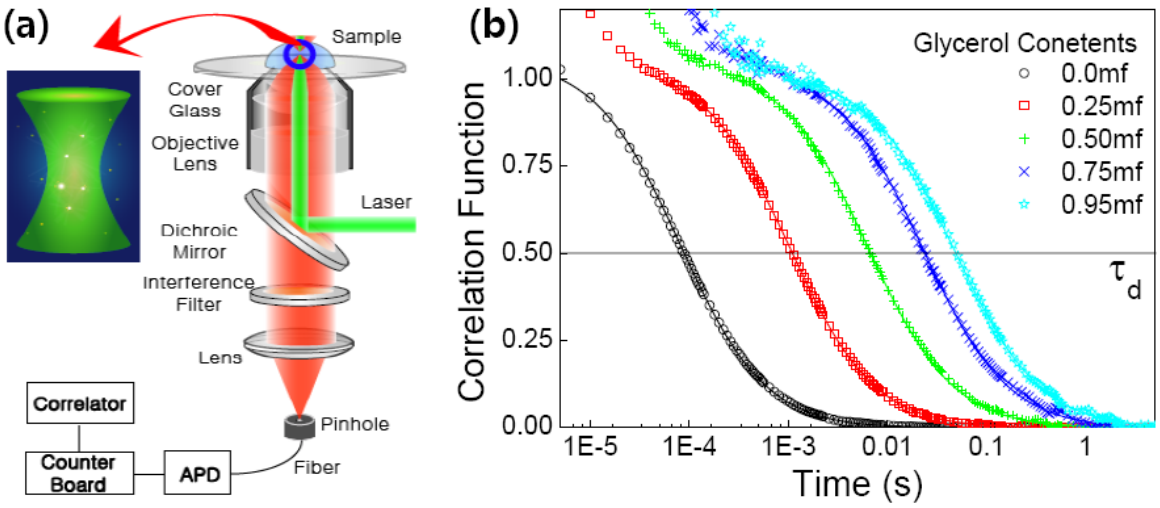

Figure 1. (a) Schematical diagram of a confocal FCS setup. Fluorescent dye molecules diffuse in and out of focal volume. (b) Normalized correlation functions of glycerol-water mixtures. The diffusion time increases with mole fraction of glycerol increases in glycerol-water mixtures (○ : pure water, □ : 0.25 mf glycerol, + : 0.50 mf glycerol, × : 0.75 mf glycerol, ☆ : 0.95 mf glycerol).

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