Single Molecule Confocal Fluorescence Lifetime Correlation Spectroscopy for Accurate Nanoparticle Size Determination

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We report on an experimental procedure in confocal single molecule fluorescence lifetime correlation spectroscopy (FLCS) to determine the range of excitation power and molecule concentration in solution under which the application of an unmodified model autocorrelation function is justified. This procedure enables fitting of the autocorrelation to an accurate model to measure diffusion length (r) and diffusion time (τ_D) of single molecules in solution. We also report on the pinhole size dependency of r and τ_D in a confocal FLCS platform. This procedure determines a set of experimental parameters with which the Stoke-Einstein equation accurately measures the hydrodynamic radii of spherical nanoparticles, enabling the determination of the particle size range for which the hydrodynamic radius by the S-E equation measures the real particle radius.

Key words : Fluorescence correlation spectroscopy, FCS, Fluorescence lifetime correlation spectroscopy, FLCS.

For characterization of individual NPs and molecules, electron microscopies such as scanning electron and transmission electron microscopy and scanning probe microscopies such as atomic force microscopy and scanning tunnelling microscopy have been employed for structural analysis at the nanometer and sub-nanometer spatial resolution. A variety of analytical spectroscopy tools such as X-ray photoelectron spectroscopy, secondary ion mass spectroscopy, and nuclear magnetic resonance have also been instrumental to assess NP chemical compositions and structural details. However, the sensitivity of these techniques is limited to ensemble-averaged measurements, and samples need to be immobilized on a substrate or in a thin film for the measurement. On the other hand, optical measurements such as dynamic light scattering and fluorescence correlation spectroscopy (FCS) allow for the non-invasive assessment of the physico-chemical properties of single molecules and NPs in solution.[1]

In summary, our approach allows for the determination of measurement conditions for proper modification to the model from which the experimental autocorrelation curve is fitted. Accordingly, r and τ_D at a specific pinhole size of the confocal microscope are accurately determined so that the Stokes-Einstein equation is applied to measure the hydrodynamic radii of spherical NPs.

We used an FLCS setup based on an inverted confocal microscope equipped with

60X oil-immersion objective (N.A 1.4, Leica). Samples were excited with 488 nm selected from a super-continuum white light laser with an 80 MHz repetition rate. This excitation was focused on the samples and fluorescent light was collected by the same objective. The fluorescent signal was separated from the scattered laser light by a dichroic mirror and focused onto an avalanche photodiode detector. The NIM output from the detector was connected to the input of a Time-Correlated Single Photon Counting module (TCSPC) and detector router for recording the time events, whereas the NIM output of the laser was also connected with the input trigger of TCSPC module. The instrument response of the entire system was about 200 ps, providing 20 ps time resolution with deconvolution. This operated in the time-tagged time-resolved (TTTR) mode, which allowed us to record the all-time events.

Alexa Fluor 488 and dragon green-labeled fluorescent beads were diluted in MilliQ water and followed by sonicating for 30 min for dispersion before the measurement. They have the nominal sizes of 50 nm, 100 nm, 190 nm, 310 nm, 520 nm, and 780 nm.

We report on an experimental procedure for determining the ranges of excitation power and concentration in confocal single molecule FLCS for which the unmodified autocorrelation model may appropriately be applied to fit the experimental autocorrelation curves. We also demonstrate that diffusion length and diffusion time are dependent upon the confocal pinhole size. These findings allow for the fitting of the autocorrelation to a proper model to accurately measure diffusion length and diffusion time of single molecules and single particles under confocal excitation volume so that the Stokes-Einstein equation is applied to accurately measure the hydrodynamic radii of spherical nanoparticles. In Fig. 1, our results show the FLCS-based Stokes-Einstein equation provides the accurate sizes of nanoparticles in the size range from 41.4 nm ± 6.8 nm to 205.6 nm ± 9.0 nm. This study provides a solid groundwork for the development of standardized experimental protocols in applying the Stokes-Einstein equation for accurate NP size measurement.

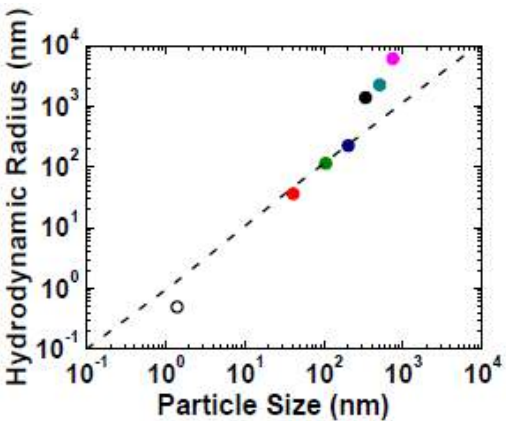

Figure 1. A plot of particle size diameters; the Alexa 488 molecule and fluorescent beads by SEM measurements vs. hydrodynamic diameter obtained by FLCS measurements and the Stokes-Einstein equation.

참고문헌

1. Bonghwan Chon, Kimberly Briggman, and Jeeseong Hwang, “Single Molecule Confocal Fluorescence Lifetime Correlation Spectroscopy for Accurate Nanoparticle Size Determination”, Phys. Chem. Chem. Phys., **16**, 13418 (2014).