DIFFUSION MEASUREMENT OF BIO-MOLECULES USING RAPID GENERATION OF BLACK HOLE IN A MOLECULAR SOLUTION BY OPTOELECTROFLUIDICS

Hyundoo Hwang and Je-Kyun Park
Department of Bio and Brain Engineering, KAIST, KOREA

ABSTRACT

We report a method for measuring diffusion coefficient of molecules based on an optoelectrofluidic platform. Optoelectrofluidic fluorescence microscopy (OFM), which is constructed with an optoelectrofluidic device and a conventional fluorescence microscope, is a useful tool for controlling and detecting local molecular concentration. In the application of a voltage at a few hundred Hertz frequency, a sudden decay of molecular concentration in the localized area, where the fluorescence excitation light was projected, occurred. After turning off the applied voltage, the dispersed molecules diffused into the molecular depletion area and the fluorescence signal was recovered. On the basis of these phenomena, we successfully measured the diffusion coefficient of various dextran molecules. This new technique based on the optoelectrofluidic platform can be a useful tool for measuring the mobility of molecules in a simple and easy way.

KEYWORDS: Optoelectrofluidics, Diffusion, Fluorescence microscopy

INTRODUCTION

Optoelectrofluidics, also called optically induced electrokinetics, is based on the electrokinetic motion of particles or fluid under an electric field induced by light [1]. By using the optically induced electrokinetic mechanisms, an image-based manipulation of several biological materials, including blood cells, oocytes, swimming bacteria, and biological molecules, has been possible. Recently, the dynamic control of local molecular concentration has been demonstrated by several frequency-dependent optoelectrofluidic phenomena such as optically induced AC electroosmosis (ACEO), dielectrophoresis (DEP) and electrostatic dipole forces [2].

Here we suggest a new method to measure the molecular diffusion in solution using an optoelectrofluidic platform based on a conventional fluorescence microscope, called optoelectrofluidic fluorescence microscopy (OFM). We demonstrated the rapid depletion of molecules within a localized area using the optically-induced electrokinetic phenomena in extremely low AC frequency range around 100 Hz with a light from a conventional fluorescence microscope.

THEORY

In the OFM, when a light for the fluorescence excitation was projected onto the photoconductive surface of the optoelectrofluidic device, the electric current resistance of the partially illuminated area was significantly decreased. Consequently, a nonuniform electric field, which induces electrokinetic motions of the fluid and elec-

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trostatic interactions among the molecules, was formed in the molecular sample solution. At the same time, we could detect the fluorescence signal from the molecules, which allows us to determine the amount of fluorophore-labeled molecules [2].

With application of AC voltage, the ACEO flow, which is converged into the illuminated area, occurred due to the optically induced tangential electric field. In addition, several mechanisms, including DEP and electrostatic interaction forces between the polarized molecules, could also be involved in the molecular behaviors and from which the molecular concentration is resulted. Consequently, rapid and significant change of molecular concentration in the illuminated area can be observed [2].

EXPERIMENTAL

Fluorescein isothiocyanate (FITC)-labeled dextran molecules of size 10 kDa, 40 kDa, and 500 kDa (Sigma–Aldrich, Milwaukee, WI) were purchased for the measurement of diffusion coefficient. Those molecules were diluted with deionized water into a concentration of 10 μM.

To fabricate a conventional optoelectrofluidic device, heavily doped hydrogenated amorphous silicon (a-Si:H), intrinsic a-Si:H, and silicon nitride were deposited sequentially on a glass substrate coated with indium tin oxide (ITO) by plasma enhanced chemical vapor deposition (PECVD) method. A sample droplet was sandwiched between the bare ITO–glass and the a-Si:H-deposited substrates.

An optoelectrofluidic device containing sample solution was put on the stage of a conventional fluorescence microscope. A fluorescence excitation light was projected onto the optoelectrofluidic device through an iris diaphragm for controlling the diameter of light pattern as 100 μm. At the same time, an AC voltage of 10 V_{pp} at 100 Hz frequency was applied across the sample solution.

RESULTS AND DISCUSSION

When we projected an excitation light pattern and applied a voltage of 10 V at 100 Hz on the optoelectrofluidic device, the FITC-dextran molecules were suddenly disappeared from the local area where the light was projected. Consequently, a dark area, where the fluorescence signal was significantly decayed, was generated in a few seconds (Figure 1). This must be due to the lateral component of electric field and the change of molecular polarization properties at the low-frequency range.

After turning off the applied voltage, temporal change of the fluorescence signal within the molecular depletion area could be determined as shown in Figure 2. The gradual recovery of the molecules at the center of the area was resulted from the diffusion of

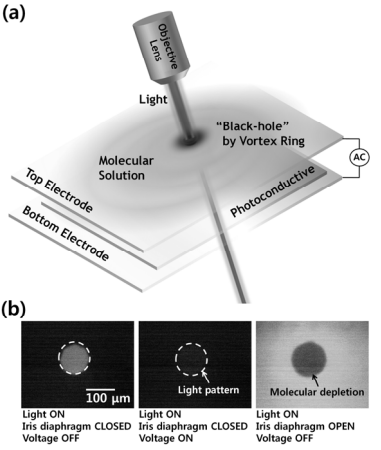

Figure 1. (a) Schematic diagram for the generation of black hole in an optoelectrofluidic platform. (b) Captured movie for rapid molecular depletion in the illuminated area.

FITC-dextran from the exterior region. Based on this approach, we could estimate the diffusion coefficient of molecules as comparing the recovery rate with a mathematical model. The measured diffusion coefficient of 10, 40, and 500 kDa FITC-dextran were 123 ± 16, 46.5 ± 6.9, and 22.6 ± 4.8 × 10^{-8} cm^2/s, respectively. These experimental values measured using OFM showed good agreement with the previously reported values measured by fluorescence recovery after photobleaching (FRAP) techniques [3, 4].

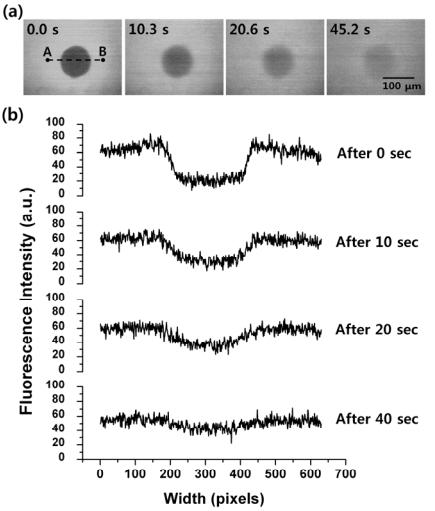

Figure 2. (a) Temporal change of the fluorescence signal after turing off the voltage. (b) Fluorescence intensity profiles along the cross section (A-B) of the illuminated area.

CONCLUSIONS

In this work, an optoelectrofluidic platform called OFM was applied to measure the molecular diffusion coefficient. We could measure the diffusion coefficient of different-sized FITC-dextran molecules using the fluorescence recovery after the optoelectrofluidic local molecular depletion. This technique would be a useful tool for analyzing electrokinetic molecular behaviours as well as studying molecular diffusion kinetics.

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