Optoelectrofluidic Platform for Manipulation and Detection of Biomolecules

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Optoelectrofluidic Platform for Manipulation and Detection of Biomolecules
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Approved by

_____________________________________
Professor Je-Kyun Park
생체분자의 조작 및 분석을 위한
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황현두

위 논문은 한국과학기술원 박사학위논문으로 학위논문심사위원회에서 심사 통과하였음.

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Abstract

This thesis presents practical applications of optoelectrofluidic platform for measurement and detection of biomolecules. Optoelectrofluidics refers to the study of the motions of particles or molecules and their interactions with surrounding fluid and electric field, which is induced or perturbed in an optical manner. Here a simple and most widely-used optoelectrofluidic platform, called optoelectrofluidic tweezers (OET), has been utilized for manipulating micro-/nanoparticles and molecules. Although many types of OET platform has been developed and many studies for programmable manipulation of various target objects using it has been reported until now, any practical applications such as measurement and detection of biological molecules has never been demonstrated. Here, in order to apply an optoelectrofluidic platform for measurement of molecular mobility and detection of human tumor markers, separation and concentration of microparticles, and dynamic control of colloidal assembly and local chemical concentration have been performed at first. In addition, the frequency-dependent behavior of micro-/nanoparticles and molecules in an optoelectrofluidic device has been investigated. On the basis of the basic
studies about the optoelectrofluidic manipulation of micro-/nanoparticles and molecules, new schemes for measuring diffusion coefficient of molecules and for conducting sandwich immunoassays have been developed. The optoelectrofluidic technologies provide a simple, rapid and easy way to measure the diffusion coefficients of various dextran molecules, and to detect human tumor marker based on surface-enhanced Raman scattering. These measurement and detection technologies based on optoelectrofluidics open a new way for simple, automated, fast, accurate and precise measurement and detection of biomolecules. In addition, this thesis provides future perspectives about practical applications of optoelectrofluidics in biology and chemistry fields.
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Nomenclature

Alphabetic letters

\( A \) \hspace{1cm} \text{Unit area}

\( C^* \) \hspace{1cm} \text{Dimensionless form of the molecular concentration}

\( C_0 \) \hspace{1cm} \text{Molecular concentration within the illuminated area}

\( C_1 \) \hspace{1cm} \text{Molecular concentration outside the illuminated area}

\( C_{\text{EDL}} \) \hspace{1cm} \text{Capacitance of the electric double layer}

\( C_{\text{liquid}} \) \hspace{1cm} \text{Capacitance of the liquid layer}

\( C_{\text{pass}} \) \hspace{1cm} \text{Capacitance of the passivation layer}

\( C_{\text{photo}} \) \hspace{1cm} \text{Capacitance of the photoconductive layer}

\( d \) \hspace{1cm} \text{Distance between two particles}

\( d_{ij} \) \hspace{1cm} \text{Unit vector in the direction from the center of \( i \)-th particle to the center of \( j \)-th particle}

\( D \) \hspace{1cm} \text{Diffusion coefficient}

\( e \) \hspace{1cm} \text{Elementary charge}

\( E \) \hspace{1cm} \text{Electric field}

\( E_t \) \hspace{1cm} \text{Tangential electric field}

\( f \) \hspace{1cm} \text{Frequency}

\( F_{\text{ACEO}} \) \hspace{1cm} \text{Hydrodynamic drag force by ACEO flow}

\( F_{\text{Coulomb}} \) \hspace{1cm} \text{Coulomb force}

\( F_{\text{DEP}} \) \hspace{1cm} \text{Dielectrophoretic force}
$F_{\text{dip}}$  Electrostatic dipole force

$F_{\text{g}}$  Gravity force

$F_{\text{net}}$  Net force

$f_{\text{CM}}$  Clausius-Mossotti factor

$j$  Imaginary number

$J_n(x)$  Bessel function of the first kind of order $n$

$k$  Boltzmann’s constant

$n_0$  Avogadro’s number

$q$  Net charge of the particle

$r$  Particle radius

$r^*$  Dimensionless form of the radial coordinate

$R$  Radius of molecular depletion area

$R_h$  Hydrodynamic radius

$\text{Re}[f_{\text{CM}}]$  Real part of the Clusius-Mossotti factor

$R_{\text{liquid}}$  Resistance of the liquid layer

$R_{\text{photo}}$  Resistance of the photoconductive layer

$t$  Thickness of the layer

$t^*$  Dimensionless form of the time

$T$  Absolute temperature

$V_{\text{ac}}$  Applied AC voltage

$v_{\text{slip}}$  Slip velocity

$z$  Electrolyte charge number
\(Z_{\text{EDL}}\)  Impedance of the electric double layer
\(Z_{\text{liquid}}\)  Impedance of the liquid layer
\(Z_{\text{pass}}\)  Impedance of the passivation layer
\(Z_{\text{photo}}\)  Impedance of the photoconductive layer

**Greek letters**

\(\alpha\)  Geometry factor
\(\alpha_n\)  Non-negative integer for calculating Bessel function zeros
\(\varepsilon^*\)  Complex permittivity
\(\varepsilon_0\)  Vacuum permittivity
\(\varepsilon_m\)  Absolute permittivity of the media
\(\varepsilon_m^*\)  Complex permittivity of the media
\(\varepsilon_p^*\)  Complex permittivity of the particle
\(\varepsilon_r\)  Relative permittivity
\(\eta\)  Fluid viscosity
\(\lambda_D\)  Debye length
\(\mu\)  Ionic mobility
\(\pi\)  Pi
\(\sigma\)  Electrical conductivity
\(\sigma_q\)  Charges contained in the Debye layer
\(\omega\)  Natural frequency of AC signal
\(\zeta\)  Zeta potential
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>2D</td>
<td>Two Dimensional</td>
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<tr>
<td>3D</td>
<td>Three Dimensional</td>
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<tr>
<td>a-Si:H</td>
<td>Hydrogenated Amorphous Silicon</td>
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<tr>
<td>AC</td>
<td>Alternating Current</td>
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<tr>
<td>ACEO</td>
<td>AC Electroosmosis</td>
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<tr>
<td>AFP</td>
<td>Alpha-Fetoprotein</td>
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<tr>
<td>AgNPs</td>
<td>Silver Nanoparticles</td>
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<tr>
<td>AuNPs</td>
<td>Gold Nanoparticles</td>
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<tr>
<td>BSA</td>
<td>Bovine Serum Albumin</td>
</tr>
<tr>
<td>CFD</td>
<td>Computational Fluid Dynamics</td>
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<tr>
<td>CM</td>
<td>Clausius-Mossotti</td>
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<tr>
<td>CMOS</td>
<td>Complementary Metal-Oxide Semiconductor</td>
</tr>
<tr>
<td>DC</td>
<td>Direct Current</td>
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<tr>
<td>DDR</td>
<td>Distance-to-Diameter Ratio</td>
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<tr>
<td>DEP</td>
<td>Dielectrophoresis</td>
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<tr>
<td>DHLA</td>
<td>Dihydrolipoic Acid</td>
</tr>
<tr>
<td>DMD</td>
<td>Digital Micro-Mirror Device</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>DP</td>
<td>4,4’-Dipyridyl</td>
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<tr>
<td>EDC</td>
<td>1-Ethyl-3-(3-Dimethylaminopropyl) Carbodiimide Hydrochloride</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<td>--------------------------------------------------</td>
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<tr>
<td>EDL</td>
<td>Electric Double Layer</td>
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<tr>
<td>EHD</td>
<td>Electrohydrodynamic</td>
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<td>ELISA</td>
<td>Enzyme-Linked Immunosorbent Assay</td>
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<tr>
<td>FCEO</td>
<td>Faradaically Coupled Electroosmosis</td>
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<tr>
<td>FITC</td>
<td>Fluorescein Isothiocyanate</td>
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<tr>
<td>hIgG</td>
<td>Human Immunoglobulin G</td>
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<tr>
<td>ICEO</td>
<td>Induced-Charge Electroosmosis</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>ITO</td>
<td>Indium Tin Oxide</td>
</tr>
<tr>
<td>IVF</td>
<td><em>in vitro</em> Fertilization</td>
</tr>
<tr>
<td>LACE</td>
<td>Light-Actuated AC Electroosmosis</td>
</tr>
<tr>
<td>LCD</td>
<td>Liquid Crystal Display</td>
</tr>
<tr>
<td>LDL</td>
<td>Lower Detection Limit</td>
</tr>
<tr>
<td>MDA</td>
<td>Molecular Depletion Area</td>
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<tr>
<td>MGITC</td>
<td>Malachite Green isothiocyanate</td>
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<tr>
<td>NHS</td>
<td>N-Hydroxysuccinimide</td>
</tr>
<tr>
<td>OET</td>
<td>Optoelectronic Tweezers</td>
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<tr>
<td>PECVD</td>
<td>Plasma-Enhanced Chemical Vapor Deposition</td>
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<tr>
<td>PR</td>
<td>Photoresist</td>
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<tr>
<td>PS</td>
<td>Polystyrene</td>
</tr>
<tr>
<td>R6G</td>
<td>Rhodamine 6G</td>
</tr>
<tr>
<td>RC</td>
<td>Resistor-Capacitor</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
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<tr>
<td>rIgG</td>
<td>Rabbit Immunoglobulin G</td>
</tr>
<tr>
<td>SERS</td>
<td>Surface Enhanced Raman Scattering</td>
</tr>
<tr>
<td>SiNₓ</td>
<td>Silicon Nitride</td>
</tr>
<tr>
<td>SiO₂</td>
<td>Silicon Dioxide</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission Electron Microscopy</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
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PART I

FUNDAMENTALS OF OPTOELECTROFLUIDICS
Part I presents fundamentals of optoelectrofluidics. Chapter 1 brings back the micromanipulation tools such as optical tweezers, magnetic tweezers, and electrokinetics, and their applications in biology and chemistry. Definition and history of optoelectrofluidics are also described before bringing the objectives of this research to light. Chapter 2 presents overview information about optoelectrofluidic platform. Essential components for constructing optoelectrofluidic platform and physical mechanisms in the device are briefly described. In this chapter, recent research progresses in the optoelectrofluidic manipulation platform for biotechnological applications are also described. Reading Part I is essential for understanding the basics of this thesis, which deals some topics related to manipulation and detection of biomolecules using an optoelectrofluidic technology extending over the following Parts.
Chapter 1: Introduction

1.1. Micromanipulation Tools for Biological Applications

Tools for non-contact micromanipulation have played a great role in several fields of science and technology. Various mechanisms, which include optical [1], magnetic [2], electrokinetic [3], and acoustic [4] forces, have been utilized to trap, transport, and sort microparticles, or to study physical properties of cells and molecules. Each technique has its own features and limitations as shown in Table 1.

Optical tweezers, which is based on the photon momentum transfer from an incident light beam to a target particle [5, 6], allows us to transport objects of a few micrometers size in three dimensional (3D) space, and to exert forces exceeding several tens of pN [7]. This powerful technique has been applied for several applications in biology [8]. For example, the measurement of forces generated by molecular motors [9-11] and the examination of mechanical properties of biological polymers [12-14] have been performed with optical tweezers. However, there are some limitations, which should be carefully considered during their use. The performance of optical tweezers is very sensitive to the conditions of light, which include optical field gradient, optical perturbation, and optical interference. The capability of parallel manipulation is limited to a small number of particles because the optical power, which is required for multiple traps, is
proportional to the number of traps [15, 16], and the field of view of the objective lens, which has high numerical aperture, limits the effective manipulation area less than $100 \times 100 \ \mu m^2$. In addition, local heating of the sample can be induced by the heat generated from the high intensity of the focused laser, which is ranged from $10^9$ to $10^{12} \ W/cm^1$.

Techniques based on the magnetic field offer some advantages over other techniques, and has been applied more various applications in biology. It is free from the problems of heating and damages due to the applied field, and is generally insensitive to the sample properties. These features permit noninvasive measurements of force and displacement in complex and heterogeneous environments such as the interior of cells [17]. Magnetic tweezers, which generally consists of a pair of permanent magnets and uses functionalized magnetic microparticles as probes, has been applied to study DNA topoisomerase [18, 19]. In typical magnetic-activated cell sorters, magnetic beads are attached to specific target cells and sorted out by applying magnetic field [20, 21]. For programmable two-dimensional (2D) manipulation of individual cells, a microelectromagnetic matrix has been utilized [22]. Recently, magnetophoretic platforms for immunoassays [23] and blood cleansing [24] have also been developed. Despite their many unique advantages, magnetic field-based techniques have also some limitations. They always require magnetic probes and strong magnetic field gradient. The robust configuration consists of permanent magnets lacks the
versatility of other manipulation techniques. Although the electromagnets can induce strong magnetic field in single cell level, they require complicated structures and high manufacturing cost, and can produce substantial heating.

AC electrokinetics has also become one of the most attractive tools for micromanipulation due to their high versatility and simple integrated configuration. Since any modification of target particles is not required as well as parallel manipulation in a large area with low power consumption is possible by patterning microelectrode array, this mechanism has been emerged as one of the most favorable tools for micromanipulation. Among various AC electrokinetic phenomena, dielectrophoresis (DEP) is one of the most frequently applied mechanisms for micromanipulation of biological materials such as DNA [25], bacteria [26], viruses [27], and cells [28]. Separation of cancer cells based on their dielectric properties was demonstrated using the balance between DEP levitation and sedimentation forces [29]. On-chip patterning of heterogeneous liver cells was also carried out using patterned microelectrodes [30]. Recently, chemical interaction forces has also been measured using DEP at single particle level [31]. However, the electrical properties of target particles and media should be considered before using these electrokinetic manipulation tools. The AC frequency-dependent characteristics of AC electrokinetic techniques also limit the applicable ranges of them in biological applications.
In 2003, Maranesi and his colleagues demonstrated two dimensional (2D) manipulation of individual cells using a programmable DEP platform based on a complementary metal-oxide semiconductor (CMOS) circuit [32]. By addressing and activating individual electrodes in the 2D electrode array, parallel manipulation of single cells, which are trapped with reconfigurable virtual DEP cages, was possible without any microfluidic and optical components. However, the integration of CMOS circuits increases the cost for manufacturing such the devices, making it less attractive for disposable applications. The wiring and interconnecting many electrodes are also remained challenging issues. To deal with these problems, optoelectronic tweezers (OET), in which the patterned microelectrodes were replaced with a photoconductive layer, was proposed by Chiou and his colleagues in 2005 [33]. By projecting a dynamic image generated from a display device, light-activated virtual electrodes are formed on the photoconductive layer, resulting in DEP motion of microparticles. On the basis of this approach, interactive manipulation of single blood cells [34], trapping motile bacteria [35], and discrimination of healthy oocytes [36] were performed for biological applications such as hematology, motility assay, and *in vitro* fertilization (IVF), respectively.
<table>
<thead>
<tr>
<th></th>
<th>Optical Tweezers</th>
<th>Magnetic Tweezers (Electromagnetic)</th>
<th>AC Electrokinetics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Minimum size of object for single trap (nm)</strong></td>
<td>250</td>
<td>500</td>
<td>100</td>
</tr>
<tr>
<td><strong>Spatial resolution (nm)</strong></td>
<td>0.1 - 2</td>
<td>5 - 10 (2 - 10)</td>
<td>$10^2$ - $10^3$</td>
</tr>
<tr>
<td><strong>Maximum transport velocity (μm/s)</strong></td>
<td>$10^2$</td>
<td>$10^3$</td>
<td>$10^3$</td>
</tr>
<tr>
<td><strong>Features</strong></td>
<td>No labeling</td>
<td>Insensitive to sample properties</td>
<td>No labeling</td>
</tr>
<tr>
<td></td>
<td>High spatial resolution</td>
<td>No damage and heating Specific interactions</td>
<td>High versatility</td>
</tr>
<tr>
<td></td>
<td>3D manipulation</td>
<td></td>
<td>Simple setup</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Easy to integrate</td>
</tr>
<tr>
<td><strong>Limitations</strong></td>
<td>Photodamage</td>
<td>Labeling</td>
<td>Sensitive to electrical properties of sample</td>
</tr>
<tr>
<td></td>
<td>Sample heating</td>
<td>No manipulation</td>
<td>Joule heating</td>
</tr>
<tr>
<td></td>
<td>Nonspecific</td>
<td>Low versatility</td>
<td>Low spatial resolution</td>
</tr>
<tr>
<td><strong>References</strong></td>
<td>[7]</td>
<td>[7]</td>
<td>-</td>
</tr>
</tbody>
</table>
1.2. Principles and History of Optoelectrofluidics

Optoelectrofluidics has appeared to deal with limitations and drawbacks of typical manipulation platforms, especially based on optics and electrokinetics. It refers to the study of the motions of particles or molecules and their interactions with surrounding fluid and electric field, which is induced or perturbed in an optical manner. This concept includes electrokinetic principles such as electrophoresis, DEP, AC electroosmosis (ACEO), and electrothermal flow, which are induced by combination of optical and electrical fields or optical-to-electrical energy transfer (See Figure 1.1 [37]).

There are three well-known methods to optically induce electrokinetic motions in a fluid as shown in Table 1.2: (1) electrothermal vortex due to the thermal gradient induced by focusing a strong laser source [38]; (2) electrophoresis due to the local increment of current density by ultraviolet exposure of indium tin oxide (ITO) [39]; and (3) OET based on a photoconductive layer deposited on a metal electrode [33].

In 1995, Mizuno et al. observed concentration of microparticles and molecules by strong vortices driven by infrared (IR) laser-induced local heating of fluid in an electric field [38]. They succeeded to stretch single DNA using the microvortex induced by a laser and an AC electric field [40]. Recently, this technique has been applied in different electrode structure, two parallel plate electrodes, and attracted attention over again [41].
Electrophoretic patterning of colloidal particles using an ultraviolet (UV) light pattern projected onto an ITO electrode was demonstrated by Hayward and his colleagues in 2000 [39]. When the UV micropattern was projected, the number of electron-hole pairs due to oxidation–reduction reaction on the interface between water and the electrode becomes increased, resulting in a perturbation of an applied electric field.

In these days, many researchers have been trying to apply a photoconductive material deposited on a plate electrode to induce a nonuniform electric field, which results in the electrokinetic motion of particles and fluids, only with a weak white light source or a conventional laser. OET is the most famous technology come under such the type of optoelectrofluidic platform. Since the first demonstration of OET in 2005, many research about and using OET in various perspectives: display, system configuration, target material, physical theory, practical function, and so on.

As a display device to generate and control light patterns, not only a digital micromirror device (DMD) [33], but also a conventional beam projector [42] and a liquid crystal display (LCD) [43] have been utilized. A lens-integrated LCD platform [34] has also been reported and widely used because of its simple configuration and powerful performance. In addition, several types of OET, which has different device structure, have been developed. 3D OET using double photoconductive layers [44], floating-
electrode OET [45], lateral field OET using single coplanar structure [46], and phototransistor-based OET [47] have been reported. Integration of microfluidic channel has also been conducted for continuous generation of liquid droplets and *in situ* manipulation [48].

Biological materials such as DNA [49, 50], polysaccharides, proteins, fluorophores [51], blood cells [34, 52], mammalian cells [46], motile bacteria [35], and oocytes [36] have been manipulated using the OET device successfully. Non-biological nanomaterials such as semiconducting nanowires [53], metal nanoparticles [54] and carbon nanotubes [55] have also been trapped and patterned. In addition, studies about operational physics in OET [56], particle-particle interactions [57], surface-particle interactions [44], optical resolution [58], and anti-fouling surface treatment [59] have been carried out. By using the OET device, various practical functions, which include manufacturing microlens array [60], separation of microparticles [61], continuous microparticle sorting [62], cell electroporation [63], and control of colloidal assembly [64], have been demonstrated.

These optoelectrofluidic platforms provide a solution for disposability and interconnection issues in the parallel manipulation of multiple particles using electrokinetic forces. Moreover, optoelectrofluidic platforms require much lower optical power and offer much larger manipulation area than typical optical mechanism such as optical tweezers. However, this
optoelectrofluidic tools still have some limitations and drawbacks, which correspond to that of electrokinetic tools such as sample dependency, low resolution, and heating problems, because they basically use electrokinetic mechanisms induced by optical methods. On the same context, any practical applications in biochemistry and clinical diagnosis, such as measurement and detection of molecules, have never been demonstrated using the optically induced electrokinetic platforms, differently from the other micromanipulation tools such as optical tweezers, magnetic tweezers, and atomic force microscopy.
Figure 1.1. Principles of optoelectrofluidics.
Table 1.2. Methods for triggering optoelectrofluidic phenomena

<table>
<thead>
<tr>
<th>Scheme</th>
<th>Year of the first demonstration</th>
<th>Light source</th>
<th>Typical electrokinetics</th>
<th>Key components</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laser-Induced Electrothermal Vortex</td>
<td>1995</td>
<td>IR laser</td>
<td>Electrothermal flow</td>
<td>Light source</td>
<td>[38]</td>
</tr>
<tr>
<td>UV-Induced Electrophoresis</td>
<td>2000</td>
<td>UV</td>
<td>Electrophoresis</td>
<td>Electrical source</td>
<td>[39]</td>
</tr>
<tr>
<td>Optoelectronic Tweezers</td>
<td>2005</td>
<td>Conventional visible light</td>
<td>Dielectrophoresis</td>
<td>Electrical source</td>
<td>[33, 61]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>AC electroosmosis</td>
<td>Mask (static pattern)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Photoconductive layer</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Display device (dynamic pattern)</td>
<td></td>
</tr>
</tbody>
</table>
1.3. Research Objectives

In this thesis, measurement and detection of biomolecules using an optoelectrofluidic manipulation platform is demonstrated. Although many types of OET platform has been developed and many studies for programmable manipulation of various target objects using it has been reported until now, none of the research could show the application of the optoelectrofluidic device in a practical manner. Here, we first apply an optoelectrofluidic platform for measurement of molecular mobility and detection of human tumor markers.

For those purpose, separation and concentration of microparticles, and dynamic control of colloidal assembly and local chemical concentration have been performed at first. We also investigate the frequency-dependent behavior of micro-/nanoparticles and molecules in an optoelectrofluidic device. In addition, theoretical studies for designing and simulating electrokinetic phenomena in an optoelectrofluidic device are performed. On the basis of the basic studies about the optoelectrofluidic manipulation of micro-/nanoparticles and molecules, new schemes for measuring or detecting biomolecules using optoelectrofluidics will be developed.

There are three approaches for measurement and detection of biomolecules using optoelectrofluidic manipulation platform. First of all, a simple method for measuring diffusion coefficient of molecules in a fluid based on an optoelectrofluidic device will be demonstrated. Secondly, a
quantitative assay of proteins using image-scanning method based on optically induced DEP of sandwich immunocomplexes will be demonstrated. Finally, image-driven sandwich immunoassays using optoelectrofluidics will be developed for simple, rapid, automated, and highly-sensitive detection of antigens. This thesis has a significant meaning in respect that this is the first practical application of the optoelectrofluidic manipulation technology in chemical and biological fields.
Chapter 2: 
Technical and Theoretical Overview

2.1. Essential Components for Optoelectrofluidic Platform

Schematic illustrations of OET, wherein a metal electrode can be replaced with an optically induced virtual electrode, are shown in Figure 2.1. In a conventional microelectronic device, microelectrodes, which can be fabricated by a conventional photolithography, are used for generating an electric field as shown in Figure 2.1(a). In an OET device, however, virtual electrodes can be optically formed on a photoconductive layer by projecting an image generated from a display as shown in Figure 2.1(b). It allows us to freely control an electric field using a programmable light pattern for micromanipulation.

To construct an optoelectrofluidic platform based on OET, a device composed of photoconductive layer and a sample solution, a light source projected to the device to induce an electric field in the sample solution by forming virtual electrodes, a display device for spatially modulating the light, and a power supply for applying a voltage are essential. Optical components such as lenses and mirrors also play a crucial role in the optoelectrofluidic system, but it depends on the display device and the light source.

ITO and hydrogenated amorphous silicon (a-Si:H) are the most widely
used materials for electrodes for the application of a voltage and a photoconductive layer deposited on a plate electrode, respectively. Since the intrinsic a-Si:H has shorter carrier diffusion length and higher optical adsorption coefficient than crystalline silicon, it is a good photoconductive material to make virtual electrode patterns of high resolution [65]. In addition, the OET device based on a-Si:H is simple and easy to fabricate using a plasma enhanced chemical vapor deposition (PECVD) method. In general, a triple layer of (i) heavily doped a-Si:H for lowering contact resistance, (ii) intrinsic a-Si:H for high photoconductivity, and (iii) silicon nitride (SiN₃) or silicon dioxide (SiO₂) for passivation was sequentially deposited onto the ITO-coated glass substrate in a single chamber reactor [43]. The fabricated OET device is shown in Figure 2.2(a) [37]. Finally, a bare ITO-coated glass substrate as a ground electrode is turned upside down and put on the fabricated photoconductive layer as sandwiching a sample solution containing target microparticles and cells with a certain gap height using spacers as shown in Figure 2.2(b). For higher photoconductivity, phototransistor has also been utilized [47]. However, the fabrication processes are too complicated and the manufacturing cost is relatively expensive.

Display device is also one of the most important components in the optoelectrofluidic platform. Sometimes, a mask with a fixed pattern [39], a diaphragm [51], or only a focused laser spot [49, 58] has been applied to
project a light onto a partial area of the photoconductive layer. However, a
display device, which is controllable by a computer, is necessary for
programmable manipulation of light-activated virtual electrodes. As a
display device, three types of device have been used as shown in Table 2.1:
(i) a DMD [33]; (ii) a beam projector [42]; and (iii) an LCD [34, 43]. In the
case of the DMD and the beam projector, they always require well-aligned
and relatively complicated optical setup for generating and focusing light
patterns, limiting the system integration for user-friendly and portable
applications.

Choi and his coworkers reported an LCD-based OET platform, named
lab-on-a-display, in 2007 [43]. There is no optical component between an
LCD and an OET device, thus a light pattern generated from an LCD is
directly transferred onto the OET device. It offers the simplest structure and
the largest manipulation area among the optoelectrofluidic platforms, which
have been reported previously. In addition, the lab-on-a-display platform is
very thin and tolerant to vibrations due to the elimination of lens and optical
alignment, providing more suitable form for portable applications. However,
the lens-less structure causes a blurred image due to the diffraction of a
light, limiting the minimum size of virtual electrodes and the performance
of particle manipulation.

To overcome those limitations of each platform, we proposed a lens-
integrated type of an LCD-based optoelectrofluidic platform [34]. In this
platform, an LCD module was installed on an illumination of a conventional microscope. A condenser lens, which is integrated in the microscope, condensed and focused the light passed through the LCD module onto the photoconductive layer of the OET device on the microscope stage. This lens-integrated type of lab-on-a-display provided much simpler and easier way to use in practice than the DMD- and the projector-based platforms, as well as much higher manipulation performances and virtual electrode resolution than the lens-less lab-on-a-display platform.

The optoelectrofluidic platform basically requires a light source, whose intensity is much lower than that for typical optical tweezers system. Therefore, we do not have to care about photonic and thermal damages of biological samples seriously. Practically, however, the type of light source is closely connected with the optical components in a whole system and the display device. When we apply a laser source, many optical components are required to project, to spatially modulate, and to focus the light onto the photoconductive surface whatever the display device used. On the other hand, in the case of a white light source such as halogen lamp, the optical components are not always required or not so complicated if we utilize an LCD as a display device. It provides higher versatility on the optoelectrofluidic system according to the target application.
Figure 2.1. Schematic illustrations of (a) a conventional microelectronic device and (b) an optoelectrofluidic platform.
Figure 2.2. Pictures of (a) the fabricated photoconductive layer and (b) the optoelectrofluidic device set on the microscope stage.
### Table 2.1. Optoelectrofluidic platforms based on optoelectronic tweezers

<table>
<thead>
<tr>
<th>Platform</th>
<th>Year of the first demonstration</th>
<th>Display device</th>
<th>Optical setup</th>
<th>Portability</th>
<th>Manipulation performance</th>
<th>Minimum pixel size (μm)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMD OET</td>
<td>2005</td>
<td>DMD</td>
<td>Complicated</td>
<td>No</td>
<td>Excellent</td>
<td>1.52</td>
<td>[33]</td>
</tr>
<tr>
<td>Projector OET</td>
<td>2005</td>
<td>Projector</td>
<td>Complicated</td>
<td>No</td>
<td>Excellent</td>
<td>5 - 35</td>
<td>[42, 50]</td>
</tr>
<tr>
<td>Lab-on-a-Display</td>
<td>2007</td>
<td>LCD</td>
<td>No</td>
<td>Yes</td>
<td>Bad</td>
<td>200</td>
<td>[43]</td>
</tr>
<tr>
<td>Lens-Integrated LCD OET</td>
<td>2008</td>
<td>LCD</td>
<td>Conventional microscope</td>
<td>Yes</td>
<td>Excellent</td>
<td>1 - 2.8</td>
<td>[34]</td>
</tr>
</tbody>
</table>
2.2. Physical Mechanisms in an Optoelectrofluidic Device

Electrokinetic mechanisms, which include electrophoresis, DEP, ACEO, electrothermal effect, and electro-orientation, are main driving force for particle manipulation using an optoelectrofluidic device. In addition, electrostatic interactions due to the polarization of dielectric particles are also observed.

Electrophoresis, the movement of charged objects in an electric field, is originated from the Coulomb force, which is defined by:

\[ F_{\text{Coulomb}} = qE, \]

where \( q \) is the net charge of the particle and \( E \) is the applied electric field. In general, most cells have functional groups, of which charge is negative at neutral pH [66]. Therefore, we can manipulate and separate different cells according to their zeta potentials by applying DC or AC electric field of extremely low frequency below about 10 Hz. Hayward et al. have patterned polystyrene (PS) microbeads based on optically induced electrophoresis using a UV light pattern which is projected onto an ITO electrode [39].

DEP, one of the most widely applied principles for optoelectrofluidic manipulation [33, 34, 36, 42-44, 46-50, 52, 60-63, 67], is the movement of dielectric objects under a nonuniform electric field driven by forces arising from the interaction between an induced electric dipole of the particle and the applied electric field [68]. The DEP force acting on a spherical particle
is given by:

\[ F_{\text{DEP}} = 2\pi r^3 \varepsilon_m \text{Re}[f_{\text{CM}}] \nabla |E|^2, \]

where \( r \) is the radius of the particles; \( \varepsilon_m \) is the permittivity of the suspending medium. \( \text{Re}[f_{\text{CM}}] \) is the real part of the Clausius-Mossotti (CM) factor which is described as below:

\[ f_{\text{CM}} = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}, \]

where \( \varepsilon_p^* \) and \( \varepsilon_m^* \) are the complex permittivities of the particle and the medium, respectively. The complex permittivity \( \varepsilon^* = 1 - j\sigma/\omega \), where \( \sigma \) is the conductivity and \( \omega \) is the angular frequency of the applied AC signal. The angular frequency is \( \omega = 2\pi f \), where \( f \) is the frequency of the AC signal. The value of \( \text{Re}[f_{\text{CM}}] \) depends on the frequency of applied AC voltage and the conductivity of particles and medium, varying between +1 and -0.5. In the optoelectrofluidic device, the particles are repelled from the light pattern, where the electric field is relatively higher than other region, if \( \text{Re}[f_{\text{CM}}] \) is negative (negative DEP). If \( \text{Re}[f_{\text{CM}}] \) is positive, the particles move toward the light pattern (positive DEP). Since most cells and molecules show the positive DEP motion in low-conductivity media, they are trapped within and moved along dynamic image patterns in the optoelectrofluidic device.

The DEP force is proportional to the volume of particle and the square of the electric field gradient. This nature of DEP force limits the rapid
manipulation of submicro-/nanoscale particles existing far from the edge of the virtual electrodes. Due to the limitation of DEP, optically induced ACEO, which is a fluidic motion generated by the motion of ions within the electric double layer due to the tangential electric field, have been applied for rapid concentration of microparticles, nanoparticles and molecules using an optoelectrofluidic device [51, 54, 61, 64]. When we project an image onto the photoconductive layer, the microparticles suspended around the image pattern are rapidly moved to the illuminated area by optically induced ACEO flow. The particles, which have been staying far away from the virtual electrodes, are also driven by the globally occurred flows. The fluids around the partially illuminated area in the optoelectrofluidic device flow along the surface of the photoconductive layer with a rectified slip velocity defined as:

\[
\langle v_{\text{slip}} \rangle = \frac{1}{2} \frac{\lambda_D}{\eta} \text{Re}[\sigma_q E^t_i],
\]

where \(\lambda_D\) is the Debye length and \(\eta\) is the fluid viscosity. The charges contained in the Debye layer (\(\sigma_q\)) and the tangential electric field (\(E_i\)) vary sinusoidally and can be evaluated as \(\sigma_q = \varepsilon_0 \zeta/\lambda_D\) and \(E_i = -\alpha \partial \zeta/\partial y\), respectively, where \(\zeta\) is the zeta potential, which is the voltage drop across the Debye layer, and \(\alpha\) is a geometry factor [69].

In addition to these electrokinetic phenomena, electrostatic interactions among the microparticles due to their induced dipole are also
observable [57]. The electrostatic interaction force, \( F_{\text{dipole}} \propto \rho_r \varepsilon_0 \text{Re}[f_{CM}]^2 E^2 \), can make the particles form a structure like perl chain by attractive forces in the direction of an electric field, and a crystalline structure with a regular distances among the particles by repulsive forces in the plane perpendicular to the electric field [70]. These electrostatic attractive and repulsive interactions can interfere with the precise control of microparticles using the optoelectrofluidic device. On the other hand, we can utilize these phenomena for several applications such as a manufacture of self-assembled micropattern structures, a study about interactions between two cells, and a bead-based immunoassay.
2.3. Equivalent Circuit Model of Optoelectrofluidic Device

The optoelectrofluidic device consists of several layers such as a photoconductive layer, an electric double layer (EDL), and a liquid layer. Therefore, the voltage drop across each layer varies against the thickness and the electrical properties of them, when a voltage was applied. The distribution of electric voltage in the device is very important for optimizing its performance for electrokinetic particle manipulation. Equivalent circuit model provides simple and easy method to design and predict the voltage drop across each layer of the optoelectrofluidic device.

The optoelectrofluidic device can be modeled in a simple resistor-capacitor (RC) circuit as shown in Figure 2.3. In the RC model, two capacitors, which correspond the passivation layer and the EDL, and two parallel RC pairs, which correspond the photoconductive and the liquid layer, are in serial form [61, 64]. Impedances, $Z$, of each component can be calculated as follows:

$$Z_{\text{liquid}} = \frac{R_{\text{liquid}}}{1 + j\omega R_{\text{liquid}} C_{\text{liquid}}},$$  \hspace{1cm} (5)

$$Z_{\text{EDL}} = \frac{1}{j\omega C_{\text{EDL}}},$$  \hspace{1cm} (6)

$$Z_{\text{pass}} = \frac{1}{j\omega C_{\text{pass}}},$$  \hspace{1cm} (7)
where $R$ and $C$ are the resistance and the capacitance, respectively. Here, the resistance of each layer, $R_{\text{liquid}}$ and $R_{\text{photo}}$, can be experimentally measured. The capacitance of each layer, $C_{\text{liquid}}$, $C_{\text{EDL}}$, $C_{\text{pass}}$, and $C_{\text{photo}}$, can be calculated as below:

$$C = \frac{\varepsilon_r \varepsilon_0 A}{t},$$

where $\varepsilon_r$ is the permittivity of liquid, $\varepsilon_0$ is the permittivity of free space, $A$ is the unit area, and $t$ is the thickness of each layer.

The voltage drop across the EDL, which is the zeta potential ($\zeta$), can be calculated through those impedances in Eqs (5) to (8) as below:

$$\zeta = V_{\text{ac}} \frac{Z_{\text{EDL}}}{Z_{\text{photo}} + Z_{\text{pass}} + Z_{\text{EDL}} + Z_{\text{liquid}}},$$

where $V_{\text{ac}}$ is the applied AC voltage. To calculate $Z_{\text{EDL}}$ in Eq (6), the thickness of the EDL, $\lambda_D$, which is called the Debye length, should be obtained, since the capacitance of the EDL is

$$C_{\text{EDL}} = \frac{\varepsilon_r \varepsilon_0 A}{\lambda_D}$$

according to the Eq (9). The Debye length in Eq (11) can be defined as

$$\lambda_D = \sqrt{\frac{\varepsilon_r \varepsilon_0 kT}{2n_0 z^2 e^2}},$$

where $\sim 2.8 \sim$
where $k$, $T$, $n_0$, $z$, and $e$ are the Boltzmann’s constant, the temperature, the Avogadro’s number, the electrolyte charge number, and the elementary charge, according to Gouy-Chapman theory [71, 72]. Here, the liquid conductivity ($\sigma$) is

$$\sigma = \mu n_0 e,$$  \hfill (13)

where $\mu$ is the ionic mobility, thus $\lambda_D$ can be calculated by experimentally measuring the liquid conductivity and applying Eqs (12) and (13).

This simple model for the optoelectrofluidic device derived by an equivalent circuit model can help the prediction of voltage drop of each layer in the device. In consequence, this mathematical model allows us to simply estimate the electrokinetic phenomena including DEP and ACEO in the device. Therefore, we can easily design an optoelectrofluidic device specific to the target applications, and optimize the experimental conditions through this equivalent circuit model.
Figure 2.3. Equivalent circuit model of optoelectrofluidic device.
PART II

OPTOELECTROFLUIDIC MANIPULATION
Part II presents optoelectrofluidic manipulation technologies, which can be fundamental tools for measurement and detection of biomolecules using optoelectrofluidics in Part III.

Separation of microparticles based on rapid and selective particle concentration by frequency-dependent optoelectrofluidic mechanisms is depicted in Chapter 3. The rapid and selective concentration of microparticles according to their size using an optoelectrofluidic platform provides important basic principles for optoelectrofluidic bead-based sandwich immunoassays, which will be depicted in Chapter 7. The part of this Chapter has been published on Lab on a Chip 9(2): 199-206 (2009).

Optoelectrofluidic control of 2D colloidal assembly in an optically induced electric field is also demonstrated in Chapter 4. The contents in this Chapter provide much information about fundamental mechanisms for behaviors of microparticles in an optoelectrofluidic device. In addition, this optoelectrofluidic colloidal assembly will be utilized to concentrate and control 2D crystal pattern for several applications such as photonic crystals and biosensors in near future (See Chapter 8). The contents of this Chapter have been published on Langmuir 25(11): 6010-6014 (2009).

Frequency-dependent behaviors of molecules in an optoelectrofluidic device are investigated in Chapter 5. Experimental investigation for optoelectrofluidic behaviors of molecules, which include polysaccharides, proteins, and fluorophores, according to the applied AC signal, their type,
and bulk concentration, has been performed in this Chapter. Temporal and spatial control of local chemical concentration in a solution droplet has also been demonstrated. The information about the optoelectrofluidic behavior of molecules at an extremely low-frequency condition will be utilized for measurement of molecular diffusion coefficient (See Chapter 6). The contents of this Chapter have been published on Analytical Chemistry 81(14):5865-5870 (2009).
Chapter 3: Optoelectrofluidic Concentration and Separation of Microparticles

3.1. Introduction

Rapid concentration and separation of microparticles including biological cells has attracted much attention in many biochemical applications and material sciences. For example, the ability to concentrate and detect the pathogenic bacteria, which are generally presented at extremely low concentration, is necessary for the early detection of them [73]. In addition, the optical components such as photonic crystals, which consist of the concentrated and assembled micro-/nanoscale particles, have also received much attention [74]. For those purposes, several mechanisms such as acoustic [75], magnetic [76, 77], hydrodynamic [78] and electrokinetic [73, 79, 80] have been suggested by many researchers. However, those systems require complicated construction, long processing time, and troublesome fluidic or electric components.

In 2005, OET for microparticle manipulation has been proposed as an alternative method for programmable electrokinetic manipulation [33]. The OET device replaced the patterned microelectrodes in conventional electrokinetic devices with a pattern-less photoconductive layer. As a result, it allows us to induce the virtual electrodes for DEP of the target particles.
by projecting a dynamic image generated from a display device onto the surface of the photoconductive layer. OET has become a powerful technology for parallel manipulation of microparticles, since unrestricted manipulation with low power consumption is possible without patterning and addressing a number of electrodes differently from other DEP-based programmable microparticle manipulators [22, 32, 81]. However, the light-induced DEP in the conventional OET device is not adapted to rapid manipulation and concentration of the particles smaller than a few micrometers. The DEP force is proportional to the cube of the particle radius and the square of the electric field gradient, limiting the DEP force acting on the submicro-/nanoscale particles existing far from the edge of the virtual electrodes [34]. In addition, it is impossible to concentrate and closely pack the particles using the light-induced DEP because of the repulsive electrostatic interactions, which are appeared by the induced-dipole of dielectric particles under the electric field, among them [57]. To conquer the limitations, an ACEO flow which is induced by a dynamic image projected from a DMD has been applied to the concentration and transportation of nanoscale particles and DNA molecules using optically-induced microfluidic vortices in the OET device [82]. According to the previous literatures about microparticle manipulator based on ACEO [80] and light-actuated AC electroosmosis (LACE) [82], the particle manipulation independent of size and electrical properties of the target
particles has been possible because the motion of fluids, not of particles, is applied for transporting particles in the ACEO-based systems. However, there is some overgeneralization that the frequency- and size-dependency of particle movements has been neglected.

Here, AC electrokinetics such as DEP and ACEO has been exploited in concert for rapid concentration and separation of microparticles using an optoelectrofluidic platform. A lab-on-a-display, which is a kind of optoelectrofluidic platform applying an LCD as a display device for generating virtual electrodes in OET [34], has been utilized for our experiments. By using the compact, integrated LCD-based OET system, we have demonstrated the size-dependent microparticle separation as well as the local concentration and assembly of microparticles originated from the image-driven AC electrokinetics. In addition, we have first characterized the frequency-dependent phenomena of the optoelectrofluidic particle concentration by the combination of several AC electrokinetics including DEP and ACEO. The reconfigurable virtual electrodes in the lab-on-a-display system are more advantageous than other microelectronic devices which apply the micro-patterned electrodes, because we can freely control the size and position of electrodes as well as the voltage conditions, which affect the particle movements and the capacity of particle concentration and separation.
3.2. Experimental

3.2.1. Device Fabrication

An OET device was fabricated with conventional PECVD method in previously reported literatures [34, 43] and in Appendix A of this thesis. Briefly, 50 nm-thickness n$^+$ doped a-Si:H and 1 μm-thickness intrinsic a-Si:H was deposited on an ITO substrate, which is a transparent conductive electrode, by turns for the photoconductive layer. Then 20 nm-thickness SiN$_x$ was also deposited on the intrinsic a-Si:H layer for the passivation. Finally, another bare ITO substrate was put upon the fabricated photoconductive substrate for the ground electrode. In this research, the height of the gap between the ground layer and the photoconductive layer, in which the target sample is located, was fixed as 30 μm with two photoresist (PR) spacers. The PR spacers were fabricated with the UV photolithographic technique on the bare ITO wafer (Samsung–Corning Precision Glass, Asan, Korea) for the ground electrode.
3.2.2. Particle Preparation

As the target particles, plain PS particles (PolySciences, PA) ranged from 500 nm to 6 μm-diameter were used for the demonstration of the rapid and selective concentration by the AC electrokinetics in the lab-on-a-display. The mixture of 1 μm and 6 μm-diameter particles, which were diluted in the de-ionized water (σ = 0.23 mS/m), were used to demonstrate the selective concentration based on their size and the AC frequency. The sample droplet was sandwiched between the ITO ground electrode and the fabricated photoconductive substrate.

3.2.3. System Configuration

A lens-integrated lab-on-a-display system was utilized for our all experiments as shown in Figure 3.1. An LCD module (L3P05S Series; Seiko Epson, Japan) for the image generation was located on the illumination of the upright microscope (Zeiss Axioskop 40; Carl Zeiss, Germany). An LCD image was transmitted to the condenser lens which is integrated in the microscope, condensing and focusing onto the photoconductive layer of the OET device on the sample stage. The bias voltage produced from a function generator (MXG-9802A; Seowon Family, Korea) was applied to the OET device. The motions of particles were captured with a charge-coupled device (CCD) camera (DS-U1; Nikon Co., Japan).
3.2.4. Measurement and Analysis

The concentrating motion of microparticles was analyzed with commercialized image processing software (NIS-Elements; Nikon Instruments Inc., NY). The frequency of the AC signal for two-dimensional crystalline assembly of microparticles was measured by scanning the AC frequency from 1 MHz to 100 Hz as observing the concentration of microparticles. When the observation in the single particle level was too difficult, like in the case of the particles smaller than 1 μm, the area where the particles were concentrated was measured. The AC frequency, at which the area of the concentrated microparticles becomes the smallest, was regarded as that for closely-packed assembly of particles.

3.2.5. Simulation

Simulation of the electric field distribution and the net forces acting on the microparticles were performed using CFD-ACE+ (ESI US R&D, Huntsville, AL).
Figure 3.1. Experimental setup for rapid and selective concentration of microparticles using an optoelectrofluidic platform.
3.3. Theory and Simulation

To predict the motion of microparticles, total net force acting on the microparticles should be calculated. For that, numerical simulation of electric field distribution in the optoelectrofluidic device was performed using commercialized computational fluid dynamics (CFD) software (CFD-ACE+; ESI US R&D, Huntsville, AL). The total net force acting on the microparticles can be presented by the superposition of three forces as follow:

\[
F_{\text{net}} = F_{\text{DEP}} + F_{\text{ACEO}} + F_g,
\]

where \(F_{\text{DEP}}, F_{\text{ACEO}},\) and \(F_g\) are the DEP force, the hydrodynamic drag force by ACEO flows, and the gravity force, respectively. \(F_{\text{DEP}}\) was calculated using simulated electric field distribution and Eq (2), and \(F_{\text{ACEO}}\) was calculated using simulated fluid velocity and Stoke’s law. Here, we assumed that a 20 \(\mu\)m-width light pattern was projected onto the center of the photoconductive layer, and a leakage of the electric current in the dark area was negligible. The liquid conductivity, \(\sigma\), and the height of liquid chamber were \(2.3 \times 10^{-6}\) S/cm and 30 \(\mu\)m, respectively. The zeta potential, which means the voltage drop across the EDL, was calculated through the equivalent circuit model as \(\zeta = 1.186\) V at 100 Hz and \(\zeta = 0.0977\) V at 10 kHz, with application of 10 \(V_{\text{pp}}\). The voltage drop across the liquid layer was only about 12.0 % of the applied voltage at 100 Hz, and about 97.4 %
at 10 kHz, according to the impedance calculation using the equivalent circuit model. The Debye length was $\lambda_D = 55.7$ nm according to the calculation using Eq (12). The other physical and electrical properties of liquid were assumed to be the same with our experimental conditions. Strictly speaking, the zeta potential and the Debye length are dependent on the applied voltage and the projected light intensity in the specific area. Therefore, those values are not spatially uniform in the optoelectrofluidic device, onto which an LCD image is projected. However, the assumption that the zeta potential and the Debye length have constant values on the illuminated area provided simple and reliable simulation using CFD-ACE+ enough to be applied for predicting the electric field distribution and the net force acting on the particles.

The simulated electric field distribution and the net force acting on 1 and 6 μm-diameter microparticles with application of a voltage of 10 $V_{pp}$ at 100 Hz are shown in Figure 3.2(a) and 3.2(b), respectively. According to the simulation, the electric field had the maximum value at the edge of the illuminated area – virtual electrode. Therefore, the velocity of the particles, which are being concentrated toward the illuminated area by optically induced ACEO vortices, was the fastest at the edge of the image pattern. The stagnation point, at which the flow velocity becomes much slower, was also appeared at the center of the light pattern, promoting the concentration of the particles. The net forces acting on both 1 and 6 μm microparticles
were toward the illuminated area by the dominant ACEO flows at 100 Hz. Therefore both particles would be concentrated into the light pattern at 100 Hz.

On the other hand, at 10 kHz, only the net force acting on 1 μm particles was in the direction toward the illuminated area by ACEO vortices, while the force acting on 6 μm ones were in the opposite direction as shown in Figure 3.3(a) and 3.3(b). Therefore, only 1 μm particles would be concentrated into the illuminated area, while 6 μm ones would be repelled from the area by the dominant negative DEP force. Based on this approach, simultaneous concentration and separation of 1 μm particles from a mixture would be possible.
Figure 3.2. Simulated electric field distribution in an optoelectrofluidic device and the calculated net force acting on (a) 1 μm and (b) 6 μm-diameter PS microparticles with application of a voltage of 10 V_{pp} at 100 Hz frequency.
Figure 3.3. Simulated electric field distribution in an optoelectrofluidic device and the calculated net force acting on (a) 1 μm and (b) 6 μm-diameter PS microparticles with application of a voltage of 10 V_{pp} at 10 kHz frequency.
3.4. Results

3.4.1. Rapid Concentration of Microparticles

When we projected an LCD image to the photoconductive layer and applied 10 Vpp at 10 kHz, the PS particles suspended around the image pattern were rapidly moved to the illuminated area by optically induced ACEO flows as shown in Figure 3.4. The particles, which were staying far away from the light patterns, were also driven by the globally induced flow. We could determine the concentration of 1 μm-diameter PS particles by measuring the temporal change of light intensity profile and the ratio of the particle-concentrated area to the illuminated area as shown in Figure 3.5(a) and 3.5(b), respectively. As time goes by, the dark area at the center of image pattern became wider because of the concentrated particles. When the particles were concentrated into the LCD image pattern by optically induced ACEO, they strongly attracted each other and became a single mass. This phenomenon made it possible to manipulate the concentrated particle group as maintaining their pattern with the projected display images (See Figure 3.6).
3.4.2. Size- and Frequency-Dependent Particle Concentration

In this work, the critical frequency of the AC signal for the 2D assembly of PS microparticles was investigated according to their size in the optoelectrofluidic platform. In the case of the 0.5 and 1 μm-diameter particles, the frequency, at which the particle-concentrated area becomes the smallest, had to be investigated, while the larger particles could be detected one by one. As shown in Figure 3.7, the critical AC frequency for the crystalline assembly of microparticles decreases as the particle diameter increases. 0.5 μm and 1 μm-diameter PS particles were closely packed at the frequencies around 10 kHz, while the 3 μm, 4.5 μm and 6 μm ones were assembled in crystalline ordering at the frequencies around 500 Hz, 300 Hz, and 100 Hz, respectively. In the case of the particles larger than 6 μm, the particle assembly was not appeared. The critical frequency was not affected by the amplitude of AC electric field and the media conductivity. As the applied voltage increases, or the conductivity decreases, the particles were concentrated faster, but the critical frequency was not changed. The measured relationship between the critical AC frequency and the diameter of the PS microparticles shows good agreement with the previous similar work, wherein two parallel electrode structure was utilized without the photoconductive layer [83].
Figure 3.4. Rapid concentration of 1 μm polystyrene particles into the LCD image pattern (dotted circle) by optically induced ACEO flows with application of a voltage of $10 \, V_{pp}$ at 10 kHz.
Figure 3.5. Temporal change of intensity profile on the dashed line A-B in Figure 3.4 and (b) the ratio of the particle-concentrated area to the illuminated area.
Figure 3.6. Manipulation of concentrated particles by controlling the projected LCD image.

Figure 3.7. Critical AC frequency for concentration and 2D assembly of microparticles against their diameter.
3.4.3. Selective Concentration of Microparticles

The frequency-dependent feature of the optoelectrofluidic forces makes it possible to control the concentration of microparticles depending on their size and the applied AC frequency. According to our experiments using a mixture of 1 μm- and 6 μm-diameter PS particles, the 1 μm ones were concentrated and assembled into the crystalline ordering at 10 kHz. At the same frequency, the 6 μm-diameter particles were repelled from the projected image patterns (See Figure 3.8, left). At 1 kHz, both 1 μm- and 6 μm particles were concentrated by optically induced ACEO, but the electrostatic particle-particle repulsions among the 6 μm particles become dominant. Consequently, the 1 μm ones filled the gap spacing between the 6 μm ones, which were located at the equilibrium position by the ACEO hydrodynamic drag force and the electrostatic repulsive interactions among them (See Figure 3.8, center). At 100 Hz, both particles were concentrated and approached to each others, but the assembly forces among the 1 μm-diameter particles was much weaker than those among the 6 μm-diameter ones. Therefore, only the 6 μm ones were concentrated into the illuminated area and closely packed in monolayer, while the 1 μm ones were pushed out from the area (See Figure 3.8, right). These results showed good agreement with the simulation results shown in Figure 3.2 and 3.3.
Figure 3.8. Microscopic pictures of 1 μm and 6 μm-diameter PS particles concentrated into the illuminated area (bright red zone) at the frequency conditions of 10 kHz (left), 1 kHz (center), and 100 Hz (right).
On the basis of these results, we could selectively concentrate the 1 μm-diameter particles into the illuminated area by adjusting the frequency of the applied AC signal as shown in Figure 3.9. At the voltage condition of 10 V\text{pp} at 10 kHz frequency, only the 1 μm-diameter particles were concentrated, while the 6 μm ones were repelled from the projected display images by negative DEP. Consequently, the 1 μm particles were spontaneously separated from the mixture.

The change of the number of 6 μm-diameter PS particles in the LCD image pattern according to the AC frequency, which was switched from 10 kHz to 100 Hz against the time, was also investigated as shown in Figure 3.10(a) and 3.10(b). Here, 9 circles, of which diameter is 50 μm, were projected at the same time. The 6 μm particles were uniformly distributed – about 10 particles per one circular pattern area – at the initial state when the image pattern was not projected. When the LCD image was projected and the voltage of 10 V\text{pp} at 10 kHz was applied, the particles were gradually repelled from the illuminated area by negative DEP forces. When the AC frequency was changed to 1 kHz, the particles were moved toward the image pattern again, as keeping a certain distance among them by the equilibrium between ACEO concentration flows and the electrostatic repulsion forces. At 100 Hz, they immediately concentrated and assembled with each other, forming crystalline structures. Therefore, the number of 6 μm-diameter PS particles in the image pattern was significantly increased.
At this time, the number of particles in one circular pattern was not uniform. This relatively large deviation was due to the competition among the image-driven concentration forces occurred by several circular patterns, which were projected at the same time into the same solution with close distances. If only one circular image pattern was projected, the number of particles would be continuously increased until most of the illuminated area was covered with the concentrated particles and saturated.
Figure 3.9. Selective concentration of 1 μm particles from a mixture of 1 μm and 6 μm PS particles with application of 10 V_{pp} at 10 kHz using the frequency dependency of optoelectrofluidics.
Figure 3.10. (a) Microscopic pictures of 1 μm and 6 μm PS particles separated by an LCD image pattern (3 × 3 circle array) at 10 kHz (left) and 100 Hz (right). (b) Change of the number of 6 μm-diameter PS particles in the LCD image pattern according to the AC frequency, which is switched from 10 kHz to 100 Hz against the time (mean ± standard deviation, n = 9).
3.5. Discussion

3.5.1. Rapid Concentration of Microparticles

According to the change of the intensity profile in Figure 3.5(a), we could have confidence that the concentrated particles (particle a in Figure 3.11) formed a monolayer on the substrate until they filled the most area of the image pattern, because the light intensity of the relatively dark area, where the particles were concentrated, were almost maintained until they filled the most area. Some residual particles (particle b, c and d in Figure 3.11), which were still being concentrated by ACEO, forced itself (particle b and c in Figure 3.11) into the outside of the concentrated particle group and pushed the particles at the center (particle a in Figure 3.11) out upward, or just whirled at the edge of the image pattern along the flow (particle d in Figure 3.11). Consequently, they formed the additional area at the center of the image pattern, where some particles were moved upward, resulting in darker transmitted image. The point that the particles formed the crystalline structures in monolayer is still open to further discussion, since it was too difficult to observe the concentrated 1 μm particles in the single particle level. However, similar results in other works support our statements [82, 84]. In addition, we could also determine the fashion of the particle concentration with the experiments applying 6 μm-diameter PS particles, which are large enough to observe in details (See the right panel of Figure...
Compared with the particle concentration using DEP or other techniques [73, 76, 78], which take tens of minutes as well as require relatively more complicated setup, it took much shorter time to concentrate 1 μm particles into extremely high density by utilizing our simple scheme. If we want to increase the capacity of the particle concentration, the programmable LCD image can be changed to be larger to concentrate larger amount of the target particles. If we want to concentrate more particles in the other place, we can move the image pattern to the target place. We can also construct microstructures by concentrating the particles with several types of image patterns and assembling them into a complete product. This technique would be used for microstructure assembly as well as rapid particle concentration.
Figure 3.11. Schematic illustration of microparticles being concentrated by optically induced ACEO flows.
3.5.2. Frequency-Dependent Particle Concentration and Assembly

As we mentioned above, the microparticles, which were concentrated into the illuminated area by optically induced ACEO, laterally assembled and formed 2D crystalline structure as shown in Figure 3.8. The self-assembly of microparticles in crystalline ordering has been detected in the previously reported works which have utilized two parallel ITO substrates [85, 86]. They proposed that the particle aggregation can be induced by induced-charge electroosmosis (ICEO) along the particle surface, which is generated from the interactions between inhomogeneous diffuse cloud and the electric field perturbed by the particle. However, the electrohydrodynamic (EHD) mechanism could account only for the particle behaviors at the AC frequency range above about 500 Hz [87]. To explain the particle behaviors which occur at the frequencies smaller than 1 kHz, Fagan et al. proposed the combination of Faradaically coupled electroosmosis (FCEO) and the EHD models [88]. At the low frequency range below a few hundred Hz, the Faradaic reaction becomes unnegligible, resulting in a lateral electric field component within the electric double layer beneath the particle. The lateral electric field acts on the diffuse layer charge cloud of the electrode, resulting in the FCEO flow [89]. To date, however, the complete theoretical model, which accounts for the colloidal assembly under the AC electric field, have never been clarified, although it has evoked much interest in the past two decades both experimentally and theoretically [83-90].
In the optoelectrofluidic device, not only the ICEO and FCEO flows along the surface of particles, but also optically induced AC electrokinetics including ACEO and DEP, the electrostatic particle-particle interactions, and the gravity force affect the particle behaviors, resulting in more complicated theoretical model. Therefore, further experimental investigations should be followed for accounting for these phenomena.

In Chapter 4, in-depth study about the optoelectrofluidic concentration and assembly of 3 μm-diameter PS particles at the AC frequency range below 1 kHz will be presented.

3.5.3. Theoretical Review of Selective Concentration of Microparticles

Although the quantitative, even qualitative, estimation of the forces acting on the particles and their motions based on the theoretical models is still open to argument, in this study, simplified estimation based on two perspectives was performed; (i) particle moving direction, which can be toward the illuminated area or not, and (ii) particle interactions, which can be attractive or repulsive.

Firstly, the combined force of ACEO and DEP can determine the particle moving direction. The ACEO flow plays a role to concentrate the particles into the illuminated area, while the negative DEP force makes the particles be repelled from the area. According to our simulation and experiments, the ACEO flow becomes dominant at the frequencies below
about 1 kHz. On the other hand, the optically induced DEP force becomes more dominant at the frequency range from 10 kHz to 1 MHz according to the numerical calculation of the CM factor and the frequency-dependent photosensitivity of the photoconductive material in our previous research [34]. However, the DEP force can be negligible in the case of the particles smaller than 1 μm because it is proportional to the square of particle radius. Therefore, we can estimate that 1 μm-diameter PS microparticles would be always concentrated into the illuminated area, while 6 μm-diameter PS microparticles, which is large enough to be affected by the DEP forces, would be repelled from the LCD image pattern at about 10 kHz and be concentrated into the image pattern at the frequency range below about 1 kHz. There are consistent with the simulation results in Figure 3.2 and 3.3.

Secondly, in the point of view about the interactions among the concentrated particles, they self-assembled in the crystalline structures at a certain optimal frequency of the AC signal by the ensemble of several mechanisms, which include FCEO, ICEO, and electrostatic interactions, at the AC frequencies below about 1 kHz. Although those mechanisms cannot completely account for the experimental results, various experimental studies have stated that the critical AC frequency for the 2D particle assembly depends on the particle size, the electrical properties of medium and particle, the temperature and many other parameters [83, 90]. According to our experimental results in Figure 3.7, 1 μm and 6 μm-
diameter PS particles were assembled and packed into the crystalline structure at about 10 kHz and 100 Hz, respectively. At 10 kHz, therefore, only 1 μm particles would be concentrated, because 6 μm particles experience the negative DEP force. At 100 Hz, both particles would be concentrated. However, according to our experimental results, only 6 μm particles were concentrated and assembled within the illuminated area at 100 Hz. This might be due to that the attraction among 6 μm particles were stronger than that among 1 μm ones. The assembly force, which is by the combinatorial force of several electrokinetics, electrostatic interactions, and gravity, acting on 1 μm particles might be weaker again at the extremely low frequencies around 100 Hz below the critical frequency, 10 kHz. Some experimental evidences derived by measuring the distances among the assembled particles will be described in Chapter 4.
3.6. Summary

We have proposed a novel scheme for rapid and selective particle concentration based on the AC electrokinetics such as DEP and ACEO, and other various mechanisms under the optically induced nonuniform AC electric field. The optoelectrofluidic manipulation allows us to rapidly concentrate and pattern micro-/nanoparticles which have a specific size by controlling the applied voltage and the projected display image without any microfluidic components. The 1 μm-diameter PS particles were completely separated from the mixture of 1 μm and 6 μm particles within 30 s with application of a voltage of 10 V_{pp} at 10 kHz, and spontaneously concentrated. In addition, the frequency-dependency of optoelectrofluidic concentration and 2D assembly of microparticles in the OET device were first characterized in the frequency range from 100 Hz to 10 kHz.

The 2D particle assembly under the optically induced AC electric field using the OET device can be applied for micro-/nanoparticles patterning, which has been issued in the colloidal science community. This novel technique can also be used in several biochemical applications using rapid concentration and separation of micro-/nanoparticles, cells and proteins as well as the manufacturing technologies for the photonic crystal components and biochemical sensors.
Chapter 4: Optoelectrofluidic Control of Colloidal Assembly

4.1. Introduction

Self-assembled colloidal structures have been used in many applications such as photonic crystals [91], colloidal lithography [92], and biochemical sensors [93]. Various methods for preparing 2D crystal structures have been proposed, including those based on capillary forces [94], electric fields [95], and the surface plasmon polariton [96]. Among these methods, the self-assembly of colloidal particles by electric-field-induced flow in a plate electrode system has attracted a great deal of interest over the past two decades [84-86]. Recently, the control of colloidal assembly has become an issue for patterning and tuning 2D crystal structures [97, 98].

Optoeletrofluidics, which refers to electrokinetic mechanisms under an optically induced electric field, has attracted a great deal of attention in several research fields because its elegant manipulation scheme depends on a light-driven virtual electrode that is reconfigurable and programmable for massively parallel processes. Optically induced DEP, which occurs in an AC electric field in the frequency range above 10 kHz, has been used to manipulate microparticles including biological cells [33-36, 46, 52]. In Chapter 3, a recent progress in separation of colloidal particles using
frequency-dependent optoelectrofluidics, which dominantly occurs in the low-frequency range below 10 kHz, has been presented [61]. In the optoelectrofluidic platform, 2D assembly of the concentrated particles, which is considered to be the result of EHD flow and electrostatic interaction forces acting on the particles, could also be observed. The possible mechanisms for optoelectrofluidic colloidal assembly (OCA) are described in detail in Section 4.3.

In this Chapter, we describe an experimental investigation of OCA as a new method for fabricating 2D colloidal crystals. The center-to-center distance among the assembled particles was analyzed according to the AC frequency. The rate of particle assembly was also measured according to the amplitude and frequency of the applied AC voltage. On the basis of the OCA, dynamic patterning of 3 μm-diameter PS particles was demonstrated using an image projected from a conventional beam projector.
4.2. Experimental

The optoelectrofluidic platform was constructed using a liquid crystal display projector (CP-S225; Hitachi, Japan) as a display device and a-Si:H deposited on an ITO electrode (Samsung–Corning Precision Glass, Korea) as a photoconductive layer. To fabricate the photoconductive layer, heavily doped a-Si:H (50 nm thick), intrinsic a-Si:H (1 μm thick), and SiNx (20 nm thick) were deposited sequentially on an ITO plate electrode using the PECVD method (See Appendix A). A bare ITO electrode was placed on the fabricated photoconductive layer with a 30 μm-thick photoresist spacer, which was fabricated through the conventional photolithography process, sandwiching a liquid sample droplet containing target microparticles. To prepare the sample droplet containing target particles for assembly, carboxylated PS particles 3 μm in diameter (PolySciences, PA) were diluted in distilled water.

Finally, an AC voltage was applied between the ITO electrode of the photoconductive layer and the bare ITO electrode, and a programmable computer image from the LCD projector was projected onto the photoconductive layer through a lens, a mirror and a condenser lens. As a consequence, a nonuniform electric field that causes several electrokinetic phenomena, including DEP and ACEO, was formed in the liquid droplet due to the significantly increased number of electron–hole pairs at the
partially illuminated surface of the photoconductive layer. The motion of colloidal particles was observed under an upright microscope (Zeiss Axioskop 40; Carl Zeiss, Germany) and recorded using a CCD camera (DS-U1; Nikon Instruments, Inc., NY). The images of assembled particles were analyzed using a conventional image analysis program (i-Solution; IMT i-Solution, Korea). The experimental setup is shown in Figure 4.1.

The simulation works were performed using CFD-ACE+ (ESI US R&D, Huntsville, AL) and all graphs were plotted using Origin 7.0 (OriginLab Corp., Northampton, MA).
Figure 4.1. Schematic illustration and experimental setup for optoelectrofluidic colloidal assembly (OCA).
4.3. Theory and Simulation

4.3.1. Mechanisms for Concentrating Particles

Mechanisms for concentrating and assembling microparticles have already been minutely carried out in Chapter 2 and 3. Briefly, optically induced electrokinetics such as DEP and ACEO, and electrostatic interactions, which depend on the applied AC frequency, affect the behavior of particles in concert. The DEP force was the most dominant at the frequencies higher than 10 kHz, while the ACEO drag force was the most dominant at the frequencies lower than 1 kHz. Nadal et al. predicted the ACEO flows in the two parallel electrodes, in which a dielectric strip laid on the bottom electrode [99]. The structure is a very similar geometry to that of a conventional optoelectrofluidic device. However, it is difficult to completely predict the particle behavior in the optoelectrofluidic device based on the model, since not only ACEO, but also other forces such as DEP and gravity affect the particle motion. The possible mechanisms for OCA are illustrated in Figure 4.2. Here, we simulated the net force, which is superposition of DEP, ACEO drag, and gravity, acting on 3 μm-diameter PS particles around a light pattern against the applied AC frequencies ranged from 100 Hz to 10 kHz.

The simulated electric field distribution and the calculated net forces acting on 3 μm particles with application of 1 kHz and 3 kHz are shown in
Figure 4.3(a) and 4.3(b), respectively. In this simulation study, we assumed that the voltage drop across the liquid layer and the EDL ($\zeta$) are varied with the applied AC frequency according to the equivalent circuit model of the optoelectrofluidic device. For example, at 1 kHz, the voltage drop across the liquid layer was only about 76.0 % of the applied voltage, and $\zeta=0.749$ V when we applied 10 V$_{pp}$. At 3 kHz, the voltage drop across the liquid layer was about 94.4 % of the applied voltage, and $\zeta=0.311$ V when we applied 10 V$_{pp}$. Here, the leakage of the applied voltage in the dark area was neglected, although there is the dark conductivity of the photoconductive layer, which was measured as about 0.1 $\mu$S/m. The conductivity was increased to 1 mS/m when a light was projected. The liquid conductivity was 0.23 mS/m and the thickness of each layer was assumed to be the same with that of the optoelectrofluidic device, which we fabricated. According to the simulation results, 3 $\mu$m particles around the virtual electrodes would be concentrated into the illuminated area by the net force in the direction toward the virtual electrode at 1 kHz, while they would not at 3 kHz. This phenomenon might be due to the canceling of the ACEO drag force by the negative DEP force at 3 kHz.

The lateral component of the net force at the position of 3 $\mu$m-diameter PS particles illustrated in the inset of Figure 4.4 (point A) was also calculated against the applied AC frequencies ranged from 100 Hz to 10 kHz as shown in Figure 4.4. Here the simulation parameters were
determined using the same processes with the processes for Figure 4.3. The calculated lateral force has the maximum value at the frequencies around 500 – 600 Hz, and negative values at the frequencies above 2 kHz. Therefore, 3 μm-diameter PS particles would be concentrated into the illuminated area at the frequencies below 2 kHz by the ACEO drag force. At the frequencies above 2 kHz, 3 μm particles would be repelled from the light pattern by dominant negative DEP, or circulated along the ACEO vortices. Consequently, we should apply an AC signal, of which frequency is lower than 2 kHz, for OCA.
Figure 4.2. Possible mechanisms for optoelectrofluidic colloidal assembly.
Figure 4.3. Simulated electric field distribution in an optoelectrofluidic device and the calculated net force acting on 3 μm-diameter PS microparticles with application of a voltage of 10 V_{pp} (a) at 1 kHz and (b) at 3 kHz.
Figure 4.4. Lateral component of the calculated net force acting on 3 μm-diameter PS microparticles at point A (inset) against the applied AC frequency with application of a voltage of 10 V_{pp}. 
4.3.2. Mechanisms for Controlling Inter-Particle Distance

When the particles are concentrated into the stagnation point, where the flow rate becomes almost zero due to the converged flows, around the center of a light pattern by ACEO, they are assembled into a crystalline structure with regular distance among them. The inter-particle distance is determined by compound action of several mechanisms such as electrostatic interactions among the particles, ICEO along the particle surface, and FCEO beneath the particles.

Electrostatic particle-particle interactions, which are generated by induced dipole of dielectric particles in an electric field, are one of the most important phenomena when we apply an optoelectrofluidic device to manipulate particles [57]. The electrostatic force acting on the polarized particle can be calculated using an equation based on the point-dipole model as below [70]:

$$F_{dip} = \frac{12\pi r^6 \epsilon_m \text{Re}[\chi_{CM}]}{d^4} \left[ d_{ij} (E_i \cdot E_j) + (d_{ij} \cdot E_i)E_j + (d_{ij} \cdot E_j)E_i - 5d_{ij} (E_i \cdot d_{ij}) (E_j \cdot d_{ij}) \right], \tag{15}$$

where $d$ is the distance between two particles, and $d_{ij}$ is the unit vector in the direction from the center of $i$-th particle to the center of $j$-th particle. The real part of CM factor ($\text{Re}[\chi_{CM}]$) is $-0.5$ in the case of PS microparticles suspended in the low-conductivity media ($\sigma \sim 10^{-4}$ S/m), thus the magnitude of the electrostatic force does not depend on the frequency of the applied voltage in our experimental conditions. The normalized electrostatic forces
acting on a particle around another particle are calculated as shown in Figure 4.5. Here, the positions of each particle were normalized by their radius. According to this mathematical model, forces acting on the particle along the line $x = 0$ are always attractive while those on the particles along the line $z = 0$ are always repulsive. These results show good agreement with the result of elsewhere literature [100]. When the particles are concentrated and assembled within the illuminated area, they typically form a monolayer structure. Therefore, they are located on the same plane, which is perpendicular to the electric field. In consequence, the repulsive interaction would dominantly affect the distances among the particles at the frequencies ranged from 100 Hz to 1 kHz, which are our experimental conditions.
Figure 4.5. Normalized electrostatic forces acting on a particle around another particle (circle); vectors (left) and magnitudes (right).
ICEO, which is driven by an electric field acting on diffuse charges induced near a polarizable surface [101], includes the electroosmotic flows along both the photoconductive surface and particle surface [102]. In this thesis, to distinguish the two electroosmotic phenomena, we called the former ACEO, and the latter ICEO. In addition, FCEO has also been proposed to explain the assembly characteristics of colloids in the low-frequency range below a few hundred hertz, in which the faradaic reaction becomes considerable [97]. Although this phenomenon has not yet been clarified with a complete mathematical model, it has been shown to be driven by the lateral electric field component within the EDL beneath the particles near the virtual electrode [88, 89]. In general, the ICEO flows are known to assemble and pack the particle on the illuminated area of the optoelectrofluidic device, while the FCEO flows are known to make the assembled particles levitate and come apart.
4.4. Results

4.4.1. Optoelectrofluidic Colloidal Assembly

When we applied AC frequency ranges below about 1 kHz, the optically induced ACEO flow could be observed. The ACEO flow converging into the light pattern formed a stagnation point at which the flow velocity approaches zero and an up-current of flow appeared, resulting in vortices around the center of the light pattern. The particles following the optically induced ACEO flow could be transported and trapped in the stagnation point at a certain AC frequency. When the colloidal particles were located in the region of stagnation, they were rapidly assembled in a crystalline structure. A 2D assembly of carboxylated PS particles 3 μm in diameter suspended in deionized water in the optoelectrofluidic device is shown in Figure 4.6. In this experiment, the OCA occurred at frequencies below 1 kHz, because the negative DEP force, which moves the particles far from the light pattern, becomes dominant at frequencies above 1 kHz according to our simulation study.
Figure 4.6. Captured microscopic pictures of optoelectrofluidic assembly of 3 μm PS particles with the application of a voltage of 10 V_{pp} at 300 Hz
4.4.2. Frequency-Dependent Change of Inter-Particle Distance

The normalized distance among the assembled particles, which is defined as the distance \((d)\) to diameter \((2r)\) ratio (DDR), according to the applied AC frequency was also measured as shown in Figure 4.7. In the frequency range above 300 Hz, the DDR of our target particles increased as the frequency increased.

4.4.3. Effect of AC Signal on Assembly Rate

We measured the assembly rate according to the frequency and amplitude of the applied ac signal, as shown in Figure 4.8(a) and 4.8(b), respectively. The number of particles assembled into the light pattern, a circle 45 μm in diameter, at 10 Vpp was measured according to the time for different frequencies ranged from 100 Hz to 1 kHz. In the relatively high frequency range (700 Hz – 1 kHz), the assembly rate increased as the frequency increased, and it decreased in the low-frequency range (~ 700 Hz). For frequencies of 100, 300, and 700 Hz, more particles were assembled into the light pattern with the application of higher voltage.
Figure 4.7. Normalized distance among the particles ($d/2r$) assembled in the illuminated area according to the AC frequency.
Figure 4.8. Number of assembled PS particles (diameter, $2r = 3 \ \mu m$) per unit time according to (a) the frequency at $10 \ \text{V}_{pp}$ and (b) the amplitude at 100, 300, and 700 Hz of the applied AC signal.
4.4.4. Image-Based Control of Colloidal Assembly

The OCA allows control of the 2D assembly of colloidal crystals with an optical method. We projected an image pattern onto the photoconductive surface of the optoelectrofluidic device with application of a voltage of 10 $V_{pp}$ at 300Hz, at which the DDR of assembled particles had the minimum value, which resulted in the production of closely packed colloidal crystals and an appropriate assembly rate. The projected images were lines 35 μm in width and circles 50 μm in diameter, produced using a computer. The 2D colloidal crystals could be successfully formed and patterned in a specific area, which can be controlled with both the image pattern and the applied AC signal (See Figure 4.9).
Figure 4.9. Image-based control of the 2D colloidal assembly in an optoelectrofluidic device.
We could also dynamically control the image pattern, which allowed optical control of the 2D crystal pattern over a large area, as shown in Figure 4.10. After projecting an image pattern consisting of two circles and waiting for the assembly of some colloidal particles around the illuminated area, we moved one circle toward the other using an interactive computer program [34]. When the two circles faced each other, the assembled colloidal particles were merged into a single colony. At the same time, they were simultaneously assembled into a stable position or dispersed by upward flow at the center of the virtual electrode. When we split the merged image pattern into two circles, the particles were also separated into two areas, and their crystal structures were rearranged. The inner state of the assembled colloidal crystals including DDR could also be controlled by tuning the applied voltage as shown in Figure 4.11. In addition, the manipulation or permanent fixation of the fabricated colloidal crystals could be achieved by controlling the image pattern or by applying a DC offset voltage, respectively.
Figure 4.10. Dynamic control of 2D colloidal assembly using an interactively controlled LCD image in an optoelectrofluidic device with application of 10 V_{pp} at 1 kHz.
Figure 4.11. Frequency-based control of inner structure of the colloidal crystals assembled by optoelectrofluidics
4.5. Discussion

4.5.1. Frequency-Dependent Change of Inter-Particle Distance

The value of DDR in Figure 4.7 might be affected by the competition between the ICEO and the electrostatic dipole forces, $F_{dip}$, which induce attraction and repulsion of particles, respectively. Both mechanisms are dependent on various parameters, including the physical properties of particles and media, electric field magnitude, frequency, and temperature [61, 83, 87, 97, 102, 103]. For the 3 μm-diameter carboxylated PS particles suspended in deionized water, the electrostatic repulsive interactions among the particles, which were concentrated into the illuminated area by the optically induced ACEO flow, became more dominant than the assembly force by ICEP as the frequency increased from 300 Hz to 1 kHz. The DDR, however, decreased as the frequency increased in the low frequency range (~ 300 Hz). This would be due to the combination of a different mechanism, FCEO, mentioned above [88]. These results showed very good agreement with those of previous studies in which the conventional two-plate electrode system was used [83, 87, 88, 97, 102, 103]. Therefore, we concluded that the mechanisms of colloidal assembly in the two-plate electrode system, such as ICEO, electrostatic interactions, and FCEO, become dominant at the stagnation point around the center of the illuminated area in the optoelectrofluidic device where the optically
induced ACEO flow is negligible.

4.5.2. Effect of AC Signal on Assembly Rate

The tendency shown in Figure 4.8(b) was very similar to that described previously whereby the assembly rate was shown to be proportional to the square of the electric field magnitude [87]; this is reasonable because the electric-field-induced flows work together for colloidal assembly. However, the results shown in Figure 4.8(a) differ from those in previous studies based on the two-plate electrode system. Liu et al. reported that the particle assembly rate decreased as the frequency increased from 300 Hz to 1 kHz and increased as the frequency increased from 100 to 300 Hz [103]. In addition, they found that the frequency of the maximum assembly rate almost corresponded to that of the minimum DDR. In this OCA, however, the assembly rate of particles 3 μm in diameter had the minimum value at 700Hz and increased as the frequency decreased to 100 Hz. These differences are because the OCA occurs in the optically induced nonuniform electric field and can be promoted and controlled by the images projected onto the photoconductive surface, whereas the colloidal assembly in previous studies was randomly generated by the perturbation of a uniform electric field by the particles themselves. In the optoelectrofluidic platform, the assembly rate is affected by the optically induced ACEO flow and the DEP force, which are important components for particle transport.
into the illuminated area starting their assembly as well as the conventional EHD assembly mechanisms including ICEO and FCEO. Due to those reasons, the experimental results are not also agreed to the simulation results for the lateral force acting on a particle around a light pattern shown in Figure 4.4, which has the maximum value at the frequency around 500 – 600 Hz. Therefore, a more complex model will be needed to clarify the colloidal assembly in the optoelectrofluidic platform.

4.5.3. Dynamic Control of Colloidal Assembly

When we dynamically controlled the 2D colloidal assembly using an LCD image (See Figure 4.10), the splitting process was easier at the frequency of 1 kHz than at 300 Hz. This phenomenon might be due to the lower attraction among the particles at 1 kHz, which can be determined from the measured DDR value in Figure 4.7. This new approach to 2D colloidal assembly can be utilized for simple fabrication and patterning of 2D colloidal crystals, which has been used in various types of applications such as biochemical sensors and colloidal lithography.
4.6. Summary

Here, 2D colloidal crystals were fabricated using optoelectrofluidic mechanisms generated in an optically induced electric field. We simulated main forces, such as DEP and ACEO drag, for optoelectrofluidic particle concentration, and investigated colloidal assembly based on the electric-field-induced flows, such as ICEO and FCEO, and electrostatic particle-particle interactions in an optoelectrofluidic device. Optoelectrofluidics allowed the control of both the assembly pattern over a large area and the distances between the assembled particles by modulating the projected light pattern and the applied voltage. This OCA can be used in several applications based on 2D colloidal crystals as a novel method for programmable colloidal assembly. Further experimental studies regarding this optoelectrofluidic approach for colloidal assembly are required to expand the significance of these investigations and establish a more accurate theoretical model for OCA.
Chapter 5:
Optoelectrofluidic Control of Local Chemical Concentration in a Fluid

5.1. Introduction

Local concentration of molecules can affect the activities and assembly of them, modulating whole chemical processes in a biological system. A dynamic control of the chemical concentration in a specifically localized region of a sample fluid with a rapid and noncontact method has been difficult, although such a technology can be valuable for several biological studies, including macromolecular crowding on protein folding kinetics [104-106] and cellular chemotaxis in a chemical gradient [107, 108]. Recently, an optical heating has been applied for the dynamic modulation of chemical concentration in an aqueous droplet due to its shrinkage and expansion [109].

Optoelectrofluidics, which is also known as optically induced electrokinetics, refers to the motion of particles or molecules and their interactions with an optically induced electric field and surrounding fluid. In 1995, this concept has been demonstrated through electrothermal vortices using an IR laser focused into an electric field [38]. In 2000, electrophoresis of colloidal particles induced by an UV light pattern projected onto an ITO electrode has also been demonstrated [39]. After that,
most of the researchers have started applying a photoconductive material deposited on a metal plate electrode to induce a nonuniform electric field, which results in electrokinetic motions of particles and fluids, only with a weak white light source or a conventional laser source. Although optically induced DEP has frequently been employed to manipulate individual microparticles, light-induced ACEO has recently attracted a great deal of attention because of its capability to rapidly manipulate nanoparticles or molecules, which are too small to manipulate using DEP proportional to the particle volume [82]. In Chapter 3 and 4, the optoelectrofluidic technologies that combined both several optically induced electrokinetics including DEP and ACEO, and electrostatic interactions has been utilized for separation, concentration, and assembly of microparticles.

In the previous studies, conventional display devices such as a DMD and an LCD have usually been applied to generate and control a light pattern and electric field gradient. However, the display-based optoelectrofluidic platform has a limitation for simultaneously detecting signals induced from the light-matter interactions, including fluorescence and Raman scattering, as manipulating them. This limitation is due to the characteristic of the optoelectrofluidic device, in which a photoconductive material is used to optically induce an electric field. However, the photoconductivity of the optoelectrofluidic device was advantageous for our objective that is dynamic modulation of chemical concentration in a
fluid. We could dynamically control the local chemical concentration using optically induced electrokinetics and electrostatic molecular interactions and detect the concentration change at the same time with a single light source for fluorescence excitation. This concept is almost the same with that described in the previous study about optically induced ACEO [82]. However, only the simple concentration of nanoparticles and DNA molecules was performed, and the in-depth investigations into the phenomena were not enough.

In this Chapter, we investigate the behavior of molecules under an optically induced electric field against various factors. Here we demonstrate a simple method for dynamic control of local chemical concentration in a sample fluid using optoelectrofluidic fluorescence microscopy (OFM), in which an optoelectrofluidic device is integrated into a conventional fluorescence microscope. The local concentration change of molecules was determined according to the applied AC signal, the type of molecules, and the initial background concentration of the sample solution. On the basis of these results, the effects of optically induced electrokinetic mechanisms and electrostatic interaction forces at the molecular level on the change of local chemical concentration were investigated. Our experimental results are quite different from the previous notion about the optoelectrofluidic concentration of molecules. In addition, a dynamic control of local concentration of macromolecules such as dextran and serum albumin,
which have often been applied as a crowding agent, was demonstrated using OFM. The frequency-dependent behavior of molecules were first investigated and applied for dynamic temporal modulation of local molecular concentration in a fluid.

5.2. Experimental

5.2.1. Device Fabrication

To fabricate an optoelectrofluidic device, glass substrates coated with an ITO were purchased from Samsung–Corning Precision Glass (Korea). Triple layers of a 50-nm-thick heavily doped a-Si:H, a 1-μm-thick intrinsic a-Si:H, and a 20-nm-thick SiNx were deposited sequentially on the ITO-coated glass substrate by the PECVD method. After the fabrication of a 30-μm-thick photoresist spacer on a bare ITO-coated glass substrate, a 10 μL droplet of sample solution was sandwiched between the bare ITO—glass substrate and the a-Si:H-deposited substrate and a wrapping wire was connected for applying a voltage. The detailed fabrication processes are presented in Appendix A.
5.2.2. Sample Preparation

Several target molecules, including fluorescein isothiocyanate (FITC)-labeled dextran, fluorescein-conjugated bovine serum albumin (BSA), fluorescein, and bisbenzimide, were purchased from Sigma–Aldrich (Milwaukee, WI). FITC-dextran was diluted with deionized water into 1, 5, 10, 30, 50, 75, and 100 μM solutions. Fluorescein-BSA, fluorescein, and bisbenzimide were diluted into 10 μM solutions. The samples were stored in an ice box to keep its temperature until injecting into the optoelectrofluidic device.

5.2.3. System Configuration

A fabricated optoelectrofluidic device was put on the stage of an epi-fluorescence microscope (BA400T; Martin Microscope Company, SC) after injecting a liquid sample containing target molecules. A light for excitation of fluorescence signal was projected from a mercury lamp through an iris diaphragm for controlling the light pattern and an objective lens for focusing the light pattern onto the photoconductive layer of the optoelectrofluidic device. At the same time, an AC voltage was applied across the liquid chamber by a function generator (AGF3022; Tektronix, OR). The fluorescence signal from the sample solution was detected by a CCD camera (DS-U1; Nikon Instruments Inc., NY) and analyzed with a conventional image analysis program (NIS-Elements; Nikon Instruments
Inc.). A schematic diagram of the OFM system, in which an optoelectrofluidic device was utilized for the dynamic control of local molecular concentration using a conventional fluorescence microscope is shown in Figure 5.1.
Figure 5.1. Schematic diagram of optoelectrofluidic fluorescence microscopy (OFM) for tuning the local molecular concentration. The cross section (A-A’) of the optoelectrofluidic device was also illustrated.
5.3. Theory

Hydrodynamic drag force by ACEO and electrostatic interactions among the molecules affect the molecular behavior in an electric field much more dominantly than DEP, because of the tiny size of molecules makes their DEP much weaker. Of course, the electrostatic interaction forces are also dependent to the size of objects. In an optoelectrofluidic device, however, the ACEO flows can concentrate the molecules into a specific area by projecting a light. When the molecules are concentrated, the electrostatic interaction forces become more significant due to the short distances among them. Because the in-depth studies about optically induced ACEO, which is presented in Eq (4), have been performed from Chapter 2 to 4, here we will discuss about the electrostatic interactions among the molecules under an optically induced electric field.

According to the previous literatures in colloidal physics, which have been reported for a long time, an AC electric field can induce the aggregation of microparticles by electrostatic interactions among them by an induced dipole of them[57, 68, 70, 100, 110]. An equation for the electrostatic interaction force acting on microparticles is presented in Eq (15), although a complete theoretical model for those phenomena is not yet available. Molecules as well as the polarizable microparticles can also be segregated in an AC electric field by a mechanism which has similar physical origin to that for the aggregation of colloids [111]. Because the
molecules generally have a huge polarizability, they are segregated at lower field strength than polymer microparticles. The optically induced ACEO flows can help the electrostatic interactions among the molecules by concentrating them into the illuminated area. The positive DEP of molecules due to their high polarizability can also help their segregation, but it would be insignificant.
5.4. Results

5.4.1. Light-Activated Molecular Concentration

When a light for the excitation of fluorescence from molecules was projected onto the optoelectrofluidic device, the number of electron-hole pairs at the partially illuminated area was significantly increased. As a consequence, a nonuniform electric field was formed in the sample solution, resulting in the optically induced electrokinetic flows and electrostatic interactions around the light pattern which induce convergence or divergence of molecules. This OFM system makes both modulation and detection of local molecular concentration easily possible by applying the light source for both fluorescence excitation and electric field activation.

When we applied a voltage and projected a light pattern to the optoelectrofluidic device, a sudden change of fluorescence signal due to the change of molecular concentration in the illuminated area was detected (See Figure 5.2). With application of a voltage of 10 $V_{pp}$ at 1 kHz, the ACEO flow was induced by the movements of closely packed ions at the electric double layer on the optically induced electrode surface due to the tangential electric field with a slip velocity defined as Eq (4). The ACEO flow converging into the illuminated area forms a stagnation point, where the flow velocity approaches zero, near the center of the area, and FITC-dextran molecules in the bulk fluid were concentrated into the point. This
local chemical concentration of molecules in the illuminated area was saturated into a specific constant value within a few seconds. Figure 5.2(b) shows the rapid change of local chemical concentration according to the amplitude of the applied voltage. The time for the change of FITC-dextran concentration was almost independent of the amplitude of the applied voltage, while the value of steady-state concentration depended not only on the amplitude of the applied voltage but also on various parameters, including the applied AC frequency, the type of molecules, and the bulk chemical concentration of the solution.

5.4.2. Frequency-Dependent Concentration Changes

Microscopic pictures of the FITC-dextran solution in the OFM for different AC frequencies from 100 Hz to 100 kHz are shown in Figure 5.3(a). All the pictures were taken at the steady state, which is about 3 s after the voltage was applied. When an AC signal of 1 kHz frequency was applied, FITC-dextran concentrated into the illuminated area with high density, forming a triangular cross-sectional profile of fluorescence intensity. As the frequency increased from 1 kHz to 10 kHz, the concentration of FITC-dextran was significantly decreased. When we increased the applied frequency to 100 kHz, there was no significant change of the chemical concentration compared to that of the no voltage condition. At 100 Hz, the local chemical concentration of FITC-dextran was significantly decreased within 3 s.
Temporal change of fluorescence signal from FITC-dextran solution for different AC frequencies such as 100 kHz, 10 kHz, and 1 kHz is shown in Figure 5.3(b). The fluorescence intensity was maintained in a certain value with application of a voltage. The fluorescence intensity profiles of FITC-dextran at 1 kHz and 10 kHz had always triangular and square shapes, respectively (See Figure 5.3(a)), although the steady-state magnitude of the fluorescence signal was changed proportionally to the amplitude of the applied AC signal as shown in Figure 5.2(b). When we turned off the applied voltage, the concentration was rapidly changed into a specific value and then slowly recovered due to the diffusion transport of target molecules as shown in Figure 5.3(b).
Figure 5.2. (a) Microscopic pictures showing the change of the chemical concentration of FITC-dextran in the illuminated region of a sample fluid after application of a voltage of 10 V<sub>pp</sub> at 1 kHz. (b) Temporal change of the chemical concentration according to the amplitude of the applied voltage at 1 kHz.
Figure 5.3. (a) Spatial and (b) temporal change of the concentration of FITC-dextran in the illuminated area against the applied AC frequency with application of a voltage of 10 V\textsubscript{pp} (black/white triangle: voltage on/off).
5.4.3. Effect of Molecular Type

We also investigated the optoelectrofluidic change of local chemical concentration with various types of molecular solutions as shown in Figure 5.4. The frequency-dependent concentration change showed a different tendency according to the type of molecules. In the case of bisbenzimide (See Figure 5.4(a)) and fluorescein, which are small fluorophores, their local concentration decreased as the AC frequency decreased from 100 kHz to 1 kHz, while the concentration of FITC-dextran (See Figure 5.4(b)) and fluorescein-BSA, which are macromolecules, had a maximum value at 1 kHz and decreased as the frequency increased to 100 kHz (See Figure 5.4(c)). In Figure 5.4(c), the fluorescence intensity was normalized to the average intensity value at the no voltage condition as a control. These results are quite different from the previous notion that the molecules are always concentrated and trapped by the optically induced ACEO vortices into the illuminated area [82].

5.4.4. Molecular Behavior Within Large Light Pattern Area

When we projected a light pattern of larger area, we could clearly observe the aggregation of molecules due to the dipole-dipole interactions with application of a voltage of 10 V_{pp} at 1 kHz (See Figure 5.5). The aggregated FITC-dextran molecules were dispersed as soon as the applied voltage turned off after about 14 s.
Figure 5.4. Microscopic pictures of (a) FITC-dextran and (b) bisbenzimide solutions against the AC frequencies from 100 Hz to 100 kHz. (c) Fluorescence intensity according to the type of molecules.
Figure 5.5. Aggregation of FITC-dextran molecules within a large light pattern due to the induced dipole of the molecules under an optically induced electric field with application of $10 \, V_{pp}$ at 1 kHz.
5.4.5. Effect of Bulk Chemical Concentration

We measured the concentration of FITC-dextran, which could be locally increased by optoelectrofluidics, according to its initial concentration, which means the bulk concentration of the sample droplet (See Figure 5.6). The maximum molecular concentration with application of a voltage of 10 $V_{pp}$ at 1 kHz frequency was proportional to the initial concentration. The local concentration of molecules converged on a specific amount with a very fast response time. The time that the molecular concentration reaches the steady state was not affected by both the magnitude and frequency of the operating voltage signal, while the steady-state concentration of the molecules and the fluorescence intensity profiles were affected.
Figure 5.6. Plot of the maximum concentration of FITC-dextran modulated by optoelectrofluidics against the bulk initial concentration of a sample droplet with application of a voltage of 10 Vpp at 1 kHz.
5.4.6. Dynamic Control of FITC-Dextran Concentration

On the basis of the frequency-dependent change of the local chemical concentration, dynamic control of the FITC-dextran concentration was performed as shown in Figure 5.7(a). As switching the frequency of the applied AC signal to 1, 10, and 100 kHz with an interval time of 10 s, the maximum fluorescence intensity was measured according to the time.

Spatial control of the local chemical concentration of FITC-dextran using OFM is also demonstrated as shown in Figure 5.7(b). With application of a voltage of 10 V_{pp} at 1 kHz, FITC-dextran molecules were rapidly concentrated into a specific area by being exposed to the light source for fluorescence excitation. When we observed the sample after moving the light pattern and opening the iris diaphragm, the molecular concentration at the localized area along the trajectory of moved light was significantly increased. Finally, the concentrated molecules were diffused out according to the time.
Figure 5.7. (a) Temporal control of the local concentration of FITC-dextran with changing the frequency and amplitude of the applied AC signal with an interval of 10 s (red/blue/green triangle: 100/10/1 kHz frequency). (b) Spatial control of the local concentration of FITC-dextran was also possible as the light pattern was controlled with application of a voltage of 10 V$_{pp}$ at 1 kHz (dotted circle: light pattern).
5.4.7. Dynamic Control of Fluorescein-BSA Concentration

Fluorescein-conjugated BSA, which is one of the most widely used crowding agents for protein folding and aggregation research [105, 106], was also applied as a target molecule to control its local concentration in a temporal and spatial manner as shown in Figure 5.8(a) and 5.8(b), respectively. The fluorescence intensity profiles of BSA against the AC frequency showed very similar tendency to that of dextran. However, the time for diffusing out of BSA was longer than that of 10 kDa dextran. It was due to the smaller diffusion coefficient of BSA \(5.1 \times 10^{-11} \text{ m}^2/\text{s}\), of which the molecular weight is 65 kDa [112].
Figure 5.8. (a) Temporal control of the local concentration of fluorescein-BSA as the frequency of the applied AC signal is changed (red/blue/green triangle: 100/10/1 kHz frequency). (b) Spatial control of the local concentration of fluorescein-BSA was also possible as the light pattern was controlled with application of a voltage of 10 V<sub>pp</sub> at 1 kHz (dotted circle: light pattern).
5.5. Discussion

5.5.1. Frequency-Dependent Concentration Change

The change of intensity profiles against the applied AC frequency was shown in Figure 5.3(a). The FITC-dextran concentration at 10 kHz lower than that at 1 kHz was due to the weaker ACEO flow around the illuminated area at the high-frequency range. The weak focusing flow, which is slightly stronger than the Brownian motion of molecules at 10 kHz, decreased the amount of concentrated molecules in the illuminated area. As a consequence, the weak dipole attraction among the molecules due to the low-volume fraction of them makes the resulting chemical concentration in the illuminated area become lower [111].

According to Figure 5.3(a), there was no significant change of FITC-dextran concentration at 100 kHz. This was due to the frequency characteristics of ACEO, which is generally activated at the low-frequency range below 10 kHz. On the other hand, the positive DEP can be activated and move the molecules at the high-frequency around 100 kHz [49]. Nevertheless, the molecular concentration in the illuminated area at 100 kHz was not much different from that at the no voltage condition in this experiment. It might be due to the weak strength of the positive DEP acting on the FITC-dextran molecules, which is comparable to the Brownian motion of the molecules. Here we applied a light source much weaker than
the previous study about optically induced DEP of DNA [49], wherein a strong laser source was applied. Therefore, the DEP force proportional to the square of the electric field gradient would become much weaker. However, we could determine the effect of the weak DEP force in the experiments for dynamic temporal modulation of the local molecular concentration using a consecutive frequency change later.

In the previous studies using the optically induced ACEO, the EHD flow always concentrated the molecules into the illuminated area [82]. According to our experimental results, however, the local chemical concentration of FITC-dextran could be decreased at a few hundred Hertz. It has been shown to be driven by the electric field-induced flow due to the lateral electric field component within the electric double layer at the low-frequency range where the Faradaic reaction becomes considerable [89]. In addition, the weaken dipole interaction forces among the molecules could also help the depletion of molecules at the illuminated area.

5.5.2. Effect of Electrostatic Interactions

From the results shown in Figure 5.3(b), we concluded that the electrostatic interactions among the molecules due to their polarization in the optically induced electric field must also affect the behavior of molecules, since the EHD flow by ACEO cannot raise these phenomena for itself. For example, the first rapid decrement of molecular concentration as soon as the voltage
is turned off, before the second slow recovery due to the diffusion, might be due to the sudden disappearance of the electric field-induced attractions between the FITC-dextran molecules. The positive DEP forces can also affect the molecular concentration, but the effect of DEP will be described later.

We could also determine the electrostatic aggregation of FITC-dextran molecules, which can be a dominant factor for molecular concentration changes, through a practical experiment as shown in Figure 5.5. This electrostatic aggregation of molecules under an electric field have also been observed using DNA molecules in a capillary tube for electrophoresis [111]. The physical mechanism of our experimental observation might be much the same with that of the previous study using DNA molecules. The electrostatic interactions among the molecules became dominant near the stagnation point, where the ACEO flow can be negligible. Therefore, the molecules concentrated by ACEO flow into the stagnation point must be aggregated by the electrostatic attraction forces with application of a voltage of 1 kHz.
5.5.3. Effect of Molecular Type

On the basis of the experimental results for four different types of molecules shown in Figure 5.4, we inferred that the size of molecules is one of the important parameters to determine the tendency of the chemical concentration change in the illuminated area at the high-frequency range above 1 kHz. The smaller molecules such as fluorophores, of which the size is approximately 1 nm [113], have a larger diffusion coefficient and smaller dipole interaction energy than relatively larger molecules such as dextran and BSA, of which the size is about 22 nm [114]. In the case of small molecules, therefore, the dispersion forces due to the Brownian motion would be more dominant than the concentration forces due to the electrostatic dipole interactions and DEP, resulting in the interference with the molecular aggregation in the illuminated area. When we applied a frequency of 100 Hz, all the molecules rapidly disappeared from the illuminated area. The mechanisms regarding the significant decrement of molecular concentration, which was independent of the molecular type, in the low-frequency range below 1 kHz is considered to have different physical origin from that in the high-frequency range above 1 kHz. The causes which induced the difference between the absolute fluorescence intensity of each molecule could not be completely determined. However, it might be caused by the difference of physical and chemical properties of each molecule.
5.5.4. Effect of Bulk Chemical Concentration

According to the bulk concentration-dependent phenomena shown in Figure 5.6, we concluded that the molecules must be in a dynamic state and not be accumulated into the illuminated area. If the molecules concentrated by the optically induced ACEO flows are accumulated, the maximum concentration must have a constant value independent of the applied voltage and the initial concentration of the sample solution. However, the concentration was saturated rapidly into a specific value, which is proportional to the initial concentration and the applied ac signal and depends on the physical and chemical properties of the molecules.

The electrostatic interaction of the molecules can also account for this concentration-dependent phenomenon. If the amount of FITC-dextran molecules in the fluid is too small, the dipole attraction among the molecules might not be strong enough to keep them in the illuminated area as well as the amount of the molecules, which exist around the light pattern and can be concentrated into it, is also insufficient [115].

5.5.5. Temporal Control of Local Chemical Concentration

In the temporal modulation of FITC-dextran concentration shown in Figure 5.7(a), the differences of the molecular concentration against the applied AC frequency increased as the amplitude of the ac signal increased from 6 $V_{pp}$ to 10 $V_{pp}$. At the voltage below 6 $V_{pp}$, the change of the fluorescence
signal due to the molecular movements was too small to detect over the signal noise, since the ACEO flow and the dipole attraction among the molecules were not enough to concentrate the molecules. The response time at the frequency for increasing molecular concentration, \textit{i.e.}, from 100 to 10 kHz and from 10 to 1 kHz, was shorter than for decreasing it, \textit{i.e.}, from 1 to 10 kHz and from 10 to 100 kHz. This was due to the diffusion of the concentrated molecules, which is a much slower transport mechanism than the optically induced ACEO flows.

According to Figure 5.7(a), the chemical concentration at the frequency of 100 kHz was also dependent on the amplitude of the applied ac signal, although the change of local chemical concentration due to the optically induced ACEO did not appear at 100 kHz according to Figure 5.3. This phenomenon was due to the effect of a weak positive DEP force, which could be induced by an optically induced nonuniform electric field [49], as mentioned above. Under the consecutive concentration and release processes, the molecules, which were already concentrated into the illuminated area at 1 and 10 kHz, would also be affected by the weak DEP force trapping them before they were diffused out from the area. Therefore, the positive DEP as well as the ACEO flows and the electrostatic interactions must affect the molecular behavior in the OFM.
5.5.6. Spatial Control of Local Chemical Concentration

We could also trap an aggregate, which is shown in Figure 5.8(b), using the optoelectrofluidic vortices. However, in this experiment, the moving velocity of the light pattern was too fast to transport the aggregate. The trapped aggregate could be manipulated, only when we moved the light pattern with much slower velocity. This phenomenon is in contradiction to the results from the previous study, which emphasized that the trapping and transport of nano-sized particles without losing them is possible using the light-induced ACEO [82]. According to these results, however, it is too difficult to trap and transport molecules without any losses because of their dynamic behavior and electrostatic interactions. We concluded that the trapping and transport of molecules using the optically induced ACEO flow is possible, but the dynamic control of local chemical concentration is the more correct function of this OFM technique. As in the case of colloidal particles, some molecules may be permanently trapped and assembled in a very thin layer on the surface of the optically induced virtual electrode, but the further studies for its verification are necessary.

5.6. Summary

Dynamic control of the local chemical concentration was demonstrated
using a simple optoelectrofluidic platform, where a conventional fluorescence microscope was combined with an optoelectrofluidic device. In this OFM system, the light source for fluorescence excitation was applied for both detection and modulation of molecular concentration in a sample fluid. We first investigated the frequency-dependent change of the local chemical concentration using the OFM. The optoelectrofluidic change of the local chemical concentration was dependent on the type of molecules and the bulk concentration as well as the applied AC signal. The investigation of dipole-dipole attraction among the molecules in the optoelectrofluidic device was also performed. Temporal control of the local molecular concentration has been demonstrated by applying the frequency-dependency of several electrokinetic mechanisms and electrostatic interactions of biomolecules such as FITC-dextran and fluorescein-BSA. Spatial control of their concentration at the localized area was also performed by controlling the light pattern.

This OFM system will be utilized as a useful tool in several applications such as molecular patterning and self-assembly as well as studies about protein folding kinetics, cellular chemotaxis, molecular aggregation, and diffusion kinetics. Moreover, it can be applied for the analysis of micro/nanoparticles and molecules based on their size, structure, and permittivity, which affect their mass transfer and electrokinetic characteristics.
PART III

OPTOELECTROFLUIDIC MEASUREMENT AND DETECTION
Part III presents optoelectrofluidic measurement and detection of biomolecules based on the experimental results and the theoretical backgrounds, which have been obtained and investigated through the previous chapters.

Optoelectrofluidic method for measurement of molecular mobility in aqueous solution based on an optoelectrofluidic platform is described in Chapter 6. This optoelectrofluidic fluorescence microscopy for measurement of molecular mobility is the first analytical tool based on the optoelectrofluidic behavior of molecules at an extremely low-frequency. This technique provides a simple and precise way to measure molecular diffusion coefficient in a solution. This Chapter has been published on Analytical Chemistry 81(21): 9163-9167 (2009).

Optoelectrofluidic sandwich immunoassays for simple, fast, and automated detection of antigens are depicted in Chapter 7. The programmable regime of optoelectrofluidics makes it possible to automatically control and accelerate immunoreactions and to perform effective washing of unbounded molecules and probe nanoparticles in a nanoliter sample droplet without any microfluidic and mechanical components. Here bead-based sandwich immunoassays have been conducted in an optoelectrofluidic platform for detection of antigens using fluorescence and surface-enhanced Raman scattering. This is the first protein detection technology based on the optoelectrofluidic technology.
Chapter 6: Optoelectrofluidic Measurement of Molecular Diffusion Coefficient

6.1. Introduction

Diffusion, which is the random motion of molecules that arises from thermal energy transferred by molecular collisions, plays a key role in the transport of molecules in biological systems. One of the most widely used methods to measure the molecular diffusion is fluorescence recovery after photobleaching (FRAP) [116]. In this technique, the recovery of fluorescence is monitored to estimate the diffusion coefficient of the molecules after bleaching of fluorophore-labeled molecules with a high-intensity laser beam. FRAP is a simple and powerful technology applicable for both in vivo and in vitro and has been extensively investigated from experimental and theoretical points of view during several decades [117-119]. However, it always requires expensive and complicated microscope equipped with high-intensity laser source for photobleaching of fluorophores. In addition, high-speed image acquisition technique should be accompanied for accurate measurements of fast diffusing molecules.

The microfluidic methods based on a capillary electrophoresis chip without photobleaching have also been reported. In those methods, the fluorescence peak variance of migrated molecules was measured after their
longitudinal diffusion for a certain time in an electric field. However, some significant problems such as adsorption of molecules onto the channel wall, Joule heating, and electrodispersion were frequently contributed. In addition, a high voltage source, miscellaneous fluidic components, and complicated optical components are always required.

In this Chapter, we suggest a new method based on optoelectrofluidics to easily measure the molecular diffusion in solution using a conventional fluorescence microscope without photobleaching, high power sources, and complicated components. The OFM introduced in Chapter 5 is applied to measure diffusion coefficients of various dextran molecules. The \( t \) test was also performed for determining the significance of our experimental values in comparison with the previously reported values measured using FRAP technology.
6.2. Experimental

6.2.1. Device Fabrication

To fabricate an optoelectrofluidic device, a 50-nm-thick heavily doped a-Si:H, an 1-μm-thick intrinsic a-Si:H, and a 20-nm-thick SiNx were deposited sequentially on a glass substrate coated with ITO (Samsung–Corning Precision Glass, Asan, Korea) by the PECVD method. A 500 nL sample droplet was sandwiched between the bare ITO-glass substrate and the a-Si:H-deposited substrate with a regular gap height of 30 μm, and a wrapping wire was connected for applying voltage. The detailed fabrication processes are presented in Appendix A.

6.2.2. Sample Preparation

For the measurement of the molecular diffusion coefficient, FITC-labeled dextran molecules of size 10, 40, and 500 kDa (Sigma-Aldrich, Milwaukee, WI) were purchased. These molecules were diluted with deionized water into a concentration of 100 μM.
6.2.3. System Configuration

The optoelectrofluidic device containing a sample solution was put on the stage of a conventional fluorescence microscope (BA400T; Martin Microscope Company, SC). A fluorescence excitation light was projected onto the optoelectrofluidic device through an iris diaphragm for controlling the diameter of the light pattern. At the same time, an AC voltage of 10 V_{pp} at 100 Hz frequency was applied across the sample solution. The fluorescence signal from the sample solution was detected by a CCD camera (DS-U2; Nikon Instruments Inc., NY) and analyzed with a conventional image analysis program (NIS-Elements; Nikon Instruments Inc.).

6.2.4. Calculation, Fitting and Statistical Analysis

Calculation of the mathematical model and fitting of experimental results to the theoretical curve based on the model were performed using Matlab 7.0 (The MathWorks Inc., MA) and Origin 7.0 (OriginLab Corp., MA). The statistical analysis such as two-sample \( t \) tests was performed and all the curves were plotted using Origin 7.0. The \( t \) tests were performed using the significance criterion of \( p \leq 0.05 \).
6.3. Theory

6.3.1. Scheme for Measurement of Molecular Diffusion

A schematic diagram of the OFM system is shown in Figure 6.1. When a light for the fluorescence excitation is projected onto the photoconductive surface of the optoelectrofluidic device, the electric current resistance of the partially illuminated area is significantly decreased. As a result, a nonuniform electric field, which induces electrokinetic motions of the fluid and electrostatic interactions among the molecules, is formed in the molecular sample solution. At the same time, we can detect the fluorescence signal from the molecules, which allows us to determine the amount of fluorophore-labeled molecules.

With application of an AC voltage, the ACEO flow was converged into the illuminated area with a slip velocity defined as Eq (4) due to the optically induced tangential electric field. In addition, several mechanisms, including DEP and electrostatic interactions between the polarized molecules, could also be involved in the molecular behaviors from which the local molecular concentration is resulted. Consequently, a rapid and significant change of molecular concentration in the illuminated area could be observed as appeared in Chapter 5.

When an AC voltage of 10 \( V_{pp} \) at 100 Hz frequency was applied to the optoelectrofluidic device, the molecules in the illuminated area were
suddenly dispersed and disappeared from the area, resulting in a sudden decay of fluorescence signal. After the applied voltage was turned off, the dispersed molecules diffused into the molecular depletion area (MDA) and the fluorescence signal was recovered. The fluorescence recovery rate was dependent on the molecular mobility, thus we could measure the diffusion coefficient of various dextran molecules based on this phenomena. After measuring the change of fluorescence intensity, we fitted the experimental values with the theoretical curves, which could be obtained by solving a partial differential equation for unsteady diffusion in cylindrical coordinates, to quantitatively figure out the diffusion coefficient of molecules.
Figure 6.1. Schematics of optoelectrofluidic fluorescence microscopy for measurement of molecular diffusion based on the optoelectrofluidic molecular depletion.
6.3.2. Unsteady Diffusion in Cylindrical Coordinates

The local molecular depletion by optoelectrofluidics results in a moleculeless space within the illuminated area. The MDA can be simply modeled as a cylinder of radius $R$, which is the same as the radius of projected light fixed by an iris diaphragm, as shown in Figure 6.2. At the initial state, when the time the voltage turned off, the concentration within and outside the MDA is uniform at $C_0$ and $C_1$, respectively. In dimensionless form, the partial differential equation for unsteady diffusion with no chemical reaction is

$$\frac{\partial C^*}{\partial t^*} = \frac{1}{r^*} \frac{\partial}{\partial r^*} \left( r^* \frac{\partial C^*}{\partial r^*} \right),$$

where $C^* = (C_i - C_0)/(C_1 - C_0)$, $r^* = r/R$, and $t^* = tD/R^2$ are the dimensionless form of the molecular concentration, the radial coordinate, and the time, respectively. Here $D$ is the diffusion coefficient of molecules. The boundary conditions are $\partial C^*/\partial r^* = 0$ at $r^* = 0$ and $C^* = 1$ at $r^* = 1$ for all $t$. The solution of Eq (16) is

$$C^* = 1 - 2 \sum_{n=1}^{\infty} \frac{J_0(\alpha_n r^*)}{\alpha_n J_1(\alpha_n)} \exp(-\alpha_n^2 t^*),$$

where $J_n(x)$ is the Bessel function of the first kind of order $n$, and $J_0(\alpha_n) = 0$. The temporal and spatial change of the molecular concentration within the MDA would be calculated based on Eq (3).
The theoretical diffusion coefficient against the molecular weight can be estimated based on the Stokes–Einstein equation,

\[ D = \frac{kT}{6\pi\eta R_h}, \]  

(18)

where \( R_h \), \( k \), and \( T \) are the hydrodynamic radius, the Boltzmann’s constant, and the absolute temperature, respectively.
Figure 6.2. Illustration of cylindrical model for molecular depletion area in an optoelectrofluidic device.
6.4. Results

6.4.1. Optoelectrofluidic Local Molecular Depletion

We first determined the local molecular depletion by optoelectrofluidic mechanisms. The molecules were suddenly depleted from the illuminated area in application of a voltage of $10 \text{ V}_{pp}$ at 100 Hz, resulting in a sudden decay of fluorescence signal as shown in parts (a) and (b) of Figure 6.3. The fluorescence intensity profile, after turning off the applied voltage and opening the iris diaphragm, was measured as shown in Figure 6.3(c).

6.4.2. Fluorescence Recovery After Molecular Depletion

After turning off the applied voltage, we could determine that the molecules, which dispersed out from the illuminated area, diffused into the empty space again, recovering the fluorescence intensity of the area as shown in Figure 6.4. The recovery rate was inversely proportional to the molecular weight of FITC-dextran. In general, the diffusion coefficient of molecules decreased as the molecular weight increased. That is, the molecular recovery into the illuminated area due to the diffusion transport was faster as the diffusion coefficient of molecules increases. Therefore, we can estimate the molecular diffusion coefficient based on the measurement of fluorescence recovery after optoelectrofluidic molecular depletion.
Figure 6.3. (a) Microscopic pictures and (b) fluorescence intensity before and after applying a voltage of 10 Vpp at 100 Hz for 40 kDa FITC-dextran in solution. (c) Fluorescence intensity profile on the line a–b in panel a.
Figure 6.4. Microscopic pictures showing the recovery of 40 kDa FITC-dextran molecules by diffusion as soon as the voltage turned off.
6.4.3. Calculation of Molecular Concentration Change

The temporal and spatial change of the fluorescence intensity profile, which means the molecular concentration, within the MDA would be calculated based on Eq (3) as shown in Figure 6.5(a). In this calculation, the diffusion coefficient of the target molecules and the light radius were assumed to be $D = 46.1 \times 10^{-8} \text{ cm}^2/\text{s}$, which is our experimental value for 40 kDa FITC-dextran, and $R = 55 \ \mu\text{m}$, which is our experimental condition, respectively.

In this study, we experimentally measured the temporal change of normalized fluorescence intensity at the center of MDA and compared with the mathematical model to estimate the diffusion coefficient of molecules. The temporal change of $C^*$ at the center of MDA could be described by Eq (3) with $r^* = 0$ condition as shown in Figure 6.5(b). As our estimation, the molecular recovery rate was proportional to the diffusion coefficient of molecules.

According to the theoretical model, the precision of measurement would become worse in this experimental condition, where the radius of MDA was fixed as 55 μm as the diffusion coefficient of the target molecules becomes larger than about $100 \times 10^{-8} \text{ cm}^2/\text{s}$. However, the operation range can be tuned by adjusting the dimension of the illuminated area or the time for voltage application, which affects the size of MDA. As the radius of MDA, $R$, increases, the rate for the temporal change of $C^*$ would be slower as shown in Figure 6.5(c). We determined these
phenomena through experiments with various $R$.

6.4.4. Measurement of Molecular Diffusion Coefficient

On the basis of this approach, the diffusion coefficient of different-sized molecules could be measured by analyzing the change of fluorescence signal by diffusion transport. We measured the temporal change of $C^*$ using 10, 40, and 500 kDa FITC-dextran solution and fitted with the theoretical model. The measured diffusion coefficients of 10, 40, and 500 kDa FITC-dextran were $125.1 \pm 7.1$, $46.1 \pm 2.9$, and $22.5 \pm 1.7 \times 10^{8}$ cm$^2$/s, respectively. Figure 6.6(a) shows the measured values from an experiment for each FITC-dextran, which is well matched with the curves from the theoretical model. In addition, we also determined the effect of $R$, which is the radius of MDA, as shown in Figure 6.6(b). As the radius of MDA increased, the rate for fluorescence recovery at the center of the area decreased. The measured diffusion coefficient was still well matched with the theoretical model. Therefore, we could obtain more precise values in the case of high mobility molecules by increasing the size of MDA to increase the measurement resolution.
Figure 6.5. (a) Calculated molecular concentration profile within the molecular depletion area (MDA) as soon as the voltage turned off. $D = 46.1 \times 10^{-8}$ cm$^2$/s and $R = 55$ μm, Temporal change of calculated molecular concentration ($C^*$) at the center of MDA ($r^* = 0$) according to (b) the diffusion coefficient of molecules when $R = 55$ μm and (c) the radius of the light pattern when $D = 46.1 \times 10^{-8}$ cm$^2$/s.
Figure 6.6. Temporal change of the normalized concentration of FITC-dextran in solution against the (a) molecular weight and (b) radius of the molecular depletion are. The fitting curves based on the theoretical model were also plotted.
6.4.5. Comparison with Literature and Theoretical Values

Table 1 shows our experimental diffusion coefficients compared to the literature and theoretical values. The diffusion coefficients reported in previous literature were measured based on FRAP techniques. The measured values showed good agreement with the previously reported values. According to a significance test, the $p$ values of 10, 40, and 500 kDa FITC-dextran were 0.38, 0.81, and 0.97, respectively. Since all the probabilities were much greater than 0.05, our method for the measurement of the molecular diffusion coefficient based on the OFM allows us to measure the mobility of molecules in solution, significant to the results in previous literature measured by FRAP techniques.

The theoretical diffusion coefficient against the molecular weight could be estimated based on the Stokes–Einstein equation, Eq (18). Here the hydrodynamic radii of 10, 40, 500 kDa FITC-dextran were about 2.3, 4.6, and 16 nm, respectively [120-122]. According to the experimental results, the experimental values for 10, 40, and 500 kDa FITC-dextran molecules were larger than the theoretical values. This might be due to the hydrodynamic radii used for calculating the theoretical diffusion coefficient, which have various values as well as do not completely reflect the real values.
Table 6.1. Diffusion coefficients for different-sized FITC-dextran molecules in solution $^a$

<table>
<thead>
<tr>
<th>Molecular weight (kDa)</th>
<th>$D_{\text{exp}}$ ($\times 10^{-8}$ cm$^2$/s)</th>
<th>$D_{\text{lit}}$ ($\times 10^{-8}$ cm$^2$/s)</th>
<th>$D_{\text{theo}}$ ($\times 10^{-8}$ cm$^2$/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>$125.1 \pm 7.1$</td>
<td>$75.7 \pm 25$ [120]</td>
<td>93.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>133 [123]</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>$46.1 \pm 2.9$</td>
<td>$51.5 \pm 2.3$ [118]</td>
<td>44.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$45.0 \pm 1.6$ [119]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$46.3 \pm 4.6$ [120]</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>$22.5 \pm 1.7$</td>
<td>$22.0$ [117]</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$23.2 \pm 1.1$ [118]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$22.4 \pm 2.6$ [119]</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Experimental data are compared to literature and theoretical values
6.5. Discussion

6.5.1. Optoelectrofluidic Local Molecular Depletion

The decay rate of the fluorescence signal was increased and saturated as the applied voltage increased to 10 V_{pp} without generation of gas bubbles by the electrolysis of the media. The 100 Hz frequency was an optimal condition at which the fluorescence decay effectively occurred and the effect of the electrophoretic motion of the charged molecules could be negligible. The time for application of the voltage was also adjusted within 3 s, since it increased the size of the MDA. In addition, if the chemical concentration of the molecular solution was too high, we could not clearly observe the optoelectrofluidic molecular depletion. It might be due to a relatively high concentration of molecules moving along the ACEO flows within the MDA and strong electrostatic forces among the polarized molecules over the ACEO flow by small intermolecular distances.

Although the origin for this decrement of molecular concentration at the low-frequency range was still not completely determined, in the opinion of many researchers, it might be due to the combination of optically induced ACEO, electrostatic intermolecular forces, and interactions between the molecules and the electric double layer [51, 115, 124]. In the case of the optically induced ACEO and the electrostatic interactions among the polarized molecules, they concentrate the molecules into the
illuminated area in the frequency range from about 1 kHz to about 100 kHz. In the low-frequency range around 100 Hz, however, an AC electroosmotic flow based on a nonzero lateral component of electric field within the electric double layer becomes significant [88] and the molecular polarization properties might be changed [115], resulting in some different aspects such as molecular dispersion that can be shown. In addition, the frequency-dependent formation of the electric double layer can also be involved in the sudden dispersion of molecules in the low-frequency ac electric field [124]. Further experimental and theoretical investigations should be followed for understanding this phenomenon more completely. Nevertheless, the obvious fact is that the photobleaching of fluorophores has never been observed in our experimental conditions. When we projected a light with an without a voltage of high-frequency above 1 kHz for a long time, about 1 h, there was not much change of the fluorescence signal in both cases.
6.5.2. Calibration Process

The initial profile of the fluorescence intensity in the real experiment was not completely the same as that in the mathematical model as shown in Figure 6.3(c). Therefore, a calibration process to adjust the initial condition of $C^*$ within the illuminated area was performed before fitting the experimental results with the curves in Figure 6.5(b). For reducing such this effort, more suitable model, not a simple cylinder model, of the initial fluorescence intensity profile made by optoelectrofluidic local molecular depletion should be established.

6.5.3. Comparison with Conventional Technologies

FRAP and fluorescence correlation spectroscopy (FCS) are well developed and widely used methods for the measurement of diffusion coefficients of fluorescence molecules in dilute solutions. FRAP uses the recovery rate of fluorescence for measuring the diffusion coefficient of the molecules after photobleaching of fluorophores on the target molecules. FCS measures spontaneous fluctuations in the fluorescence signal, which comprise particle movements, conformational changes, chemical or photophysical reactions. These technologies provide very powerful ability to measure molecular mobility both in vivo and in vitro. However, they always require high-power lasers, high-speed camera, and complicated optical components. FRAP stands in need of photobleaching of fluorophores in addition.
Compared to these optical methods, this optoelectrofluidic technique, which requires no high-power lasers, no high-speed camera, no photobleaching, no fluidic components, and a few optical components, provides an easier and simpler way to measure the molecular diffusion coefficient in solution. In addition, tuning of optimal operation range for more accurate measurement is easily possible by controlling the light pattern. However, the optoelectrofluidic method requires an electrical source and dependent to the media conductivity.

All these methods are based on the fluorescence, thus always require fluorophores to label the target molecules. To deal with this limitation, non-labeling technologies such as Raman scattering could also be applicable by integrating with the optoelectrofluidic technology.
6.6. Summary

In this Chapter, we applied an optoelectrofluidic platform called OFM to measure the molecular diffusion coefficient. We could successfully measure the diffusion coefficient of FITC-dextran molecules in solution based on the fluorescence recovery after the optoelectrofluidic local molecular depletion. We constructed a simple mathematical model to explain the temporal change of molecular distribution and exploited it to estimate the diffusion coefficient. The diffusion coefficients of 10, 40, and 500 kDa FITC-dextran measured using the OFM were 125.1 ± 7.1, 46.1 ± 2.9, and 22.5 ± 1.7 × 10⁻⁸ cm²/s, respectively. These experimental values were significant to the previously reported values measured by FRAP techniques and the theoretical values calculated from the Stokes-Einstein equation. This is the first demonstration of diffusion measurement based on optoelectrofluidics, which has been applied for a practical application in analytical science. This new method will provide a simple and easy way to determine the molecular mobility in solution based on optoelectrofluidics.
Chapter 7: Optoelectrofluidic Immunoassays for Detection of Human Tumor Markers

7.1. Introduction

Immunoassay, which is based on a specific interaction between an antigen and a complementary antibody, has become an important analytical tool for identifying and quantifying a specific substance in a liquid sample. In several fields such as clinical diagnosis, biochemical analysis, and environmental monitoring, many kinds of techniques, such as fluorescence, chemiluminescence, electrochemical methods, surface plasmon resonance, and enzyme-linked immunosorbent assay (ELISA), have been utilized to measure the antigens binding to its complementary antibodies. Recently, surface-enhanced Raman scattering (SERS) has also been applied to the immunoassays [125-127]. Among several kinds of SERS-based immunoassay methods, a sandwich immunoassay using metal nanoparticles labeled by a Raman-active probe has been most widely used because of its remarkable SERS-enhancement ability and good biocompatibility. Here, a sandwich immunocomplex is formed by immunoreaction between an antigen captured by antibodies immobilized on a solid substrate and probe-labeled metal nanoparticles. Then the antigen concentration can be indirectly determined by measuring the Raman intensity of the probe-
labeled metal nanoparticles, which constitute the immunocomplexes in ‘sandwich’ form.

For all the measurement techniques for the immunoassay including the SERS-based methods, this heterogeneous strategy always requires repetitive washing steps for separating antibody-bound and free proteins. In addition, a long incubation time is required for antibody-antigen binding reactions, which is limited by diffusion transport of molecules or nanoparticles. As a consequence, the manually conducted processes of a typical heterogeneous immunoassay, which involves multiple cycles of incubation and washing steps, usually take several hours and are labor-intensive. To deal those problems of a traditional immunoassay, many kinds of fully-automated immunoassay analyzers, which can perform a various tests on a series of samples and process several batches simultaneously, have been commercialized. The automated systems usually take several tens of minutes for performing all steps including sample dilution, dispensing, incubation, washing, and reading without manual handling of liquids except during the step for sample injection. However, the commercialized immunoassay instruments still have some limitations that they requires massive and complicated constructions of robotic components and several hundreds of microliter sample volumes. Recently, microfluidic devices have also been applied for simple and rapid immunoassays in miniaturized system with small sample volumes [128-130]. The
microfluidic immunoassay platforms, however, also require troublesome fluidic components, and yield large amount of dead volumes and many disposables.

In this Chapter, a new immunoassay platform is developed for simple, rapid and automated detection of antigens using the light-activated particle manipulation technology. By applying a conventional OET device to control antibody-conjugated PS microspheres and probe-labeled silver nanoparticles (AgNPs), a SERS-based sandwich immunoassay of human tumor marker, alpha-fetoprotein (AFP), could be automatically performed using a programmed image from a conventional LCD module. A fluorescence-based assay using biotinylated rabbit immunoglobulin G (rIgG) as a target molecule was also performed to ascertain whether the free probe nanoparticles can be efficiently washed out by optoelectrofluidic selective concentration of immunocomplexes or not. This optoelectrofluidic immunoassay does not require any fluidic components and manual handling of liquids for all steps except for sample injection. Moreover, it requires only submicroliter sample droplet as a total specimen, and takes only several minutes to complete one assay cycle.
7.2. Experimental

7.2.1. Device Fabrication

A sample droplet, whose total volume is 500 nL (volume ratio of AFP antigens : PS microspheres : AgNPs = 1: 1: 1), was placed in a 30 µm-height liquid chamber of the optoelectrofluidic device, which consists of a bare ITO electrode and a photoconductive electrode. The photoconductive electrode was fabricated by sequential deposition of three layers: (1) a 50 nm-thick heavily doped a-Si:H; (2) an 1 µm-thick intrinsic a-Si:H, and (3) a 20 nm-thick silicon nitride on an ITO-coated glass substrate (Samsung–Corning Precision Glass, Korea) using a plasma enhanced chemical vapor deposition. The device surfaces were treated with 0.1% bovine serum albumin solution for about 30 min to reduce nonspecific adsorption of microspheres onto the device surface. Lastly, an AC voltage of 10 V_{pp} produced from a function generator (AFG310; Tektronix, OR) was applied across the ITO electrodes. The detailed fabrication processes are presented in Appendix A.
7.2.2. Sample Preparation

Normal rIgG-biotin was obtained from Santa Cruz Biotechnology, Inc (Santa Cruz, CA). Carboxylated fluorescent (Max. Ex/Em: 441/486) PS microspheres with diameter of 6 µm and neutravidin-coated fluorescent (Max. Ex/Em: 580/605) PS nanoparticles with diameter of 40 nm were purchased from PolySciences, Inc. (Warrington, PA) and Molecular Probes, Inc. (Eugene, OR), respectively.

After twice washing of PS microspheres with distilled water, for the activation of other -COOH terminal groups on the polystyrene microspheres, 10 µL of 10 mM N-hydroxysuccinimide (NHS) and 10 µL of 10 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were added to 470 µL of 10^8 particles/mL PS microspheres and allowed to react for 20 min. Then, 10 µL of 1.0 mg/mL monoclonal rIgG antibody (excess amount) was added to NHS-activated PS beads and reacted overnight at 4 °C. Here, unreacted antibodies were washed out by twice centrifuging, and the final PS microspheres with monoclonal rIgG antibody were redispersed in distilled water.

For targeting AFP antigens, PS microspheres and SERS-active AgNPs were functionalized with monoclonal and polyclonal AFP antibodies, respectively. Here, 10 µL of 1.0 mg/mL monoclonal AFP antibody (excess amount) was added to NHS-activated PS beads and reacted overnight at 4 °C. Lastly, unreacted antibodies were washed out by twice centrifuging,
and the final PS microspheres with monoclonal AFP antibody were redispersed in distilled water.

Silver colloids were prepared by the method reported by Leopold and Lendl [131]. Here, silver nitrate was reduced by hydroxylamine hydrochloride. The detailed procedure for the preparation of AgNPs has been reported elsewhere [132]. Briefly, 5 mL of $3.0 \times 10^{-2}$ M hydroxylamine hydrochloride was dissolved in 84 mL of triply distilled water and then 1 mL of $3.0 \times 10^{-1}$ M sodium hydroxide was added to maintain an alkaline pH. Next, 10 mL of $2.0 \times 10^{-2}$ M silver nitrate solution was added dropwise to the solution with continuous stirring. The solution was continuously stirred for an additional 1 day. UV/Vis spectroscopy and transmission electron microscopy (TEM) were used to characterize the particle size of the produced colloids. The average particle size was determined to be 40 ± 10 nm.

To use these AgNPs as SERS-active probes, malachite green isothiocyanate (MGITC) was adsorbed onto the surface of the AgNPs. An amount of 1 μL of $5 \times 10^{-5}$ M MGITC was added to 1 mL of AgNPs, and the mixture was reacted for 1 h under stirring. Then, dihydrolipoic acid (DHLA) was used for the antibody conjugation. The two -SH terminal groups of DHLA were cleaved and chemically bonded to the silver surface. An amount of 2 μL of 5.0 mM DHLA was added to 1 mL of dye-adsorbed
AgNPs and allowed to react for 1 h. Excess nonspecific binding mercaptoethanol in solution was removed by centrifuging the solution, and the precipitate was washed twice with distilled water and resuspended.

For the activation of other –COOH terminal groups, 1 μL of 1.0 mM EDC and 1 μL of 1.0 mM NHS were added and allowed to react for 20 min. Finally, 1 μL of 1.0 mg/mL polyclonal AFP antibody (excess amount) was added to NHS-activated silvernanoparticles and reacted for overnight at 4°C. Unreacted NHS groups on the surface of the AgNPs were deactivated with 1 μL of 1.0 mM ethanolamine for 2 h. Nonspecific binding chemicals and antibodies were removed by centrifuging, and the final nanoparticles were washed twice with distilled water [133].
7.2.3. System Configuration

Experimental setup for the optoelectrofluidic immunoassays is shown in Figure 7.1(a). A conventional inverted fluorescence microscope (IX71; Olympus Corp., Japan) was applied to construct the optoelectrofluidic immunoassay system. A monochromatic LCD module in a conventional projector (CP-S225; Hitachi, Japan) was mounted under the illumination lamp to generate a programmed image pattern for controlling an electric field. The LCD image was projected onto the photoconductive layer of the device through a condenser lens integrated into the microscope. The optoelectrofluidic device containing a sample droplet was put on the microscope stage as the transparent ITO electrode faces to the objective lens, since the photoconductive layer absorbs or blocks an incident laser beam and Raman scattering. The pictures of the experimental setup are shown in Figure 7.1(b).
7.2.4. SERS Detection

SERS measurements were performed using home-made Raman microscope system. An inverted microscope IX71 (Olympus Corp.) was modified for sensitive Raman scattering detection. A Coherent, Ar-Kr laser (Innova70C spectrum) operating at $\lambda = 647.4$ nm was used as the excitation source with a laser power of 5 mW. Raman scattering was collected using a CCD camera at a spectral resolution of 2 cm$^{-1}$. A Princeton instrument, int. Spectra 2500i was used as a monochrometer. An additional CCD camera was fitted to an optical microscope to obtain optical images. Sandwich immunocomplexes in an optoelectrofluidic device were collected using a 20× objective lens was used to focus a laser spot on the glass. All of the Raman spectra reported here were collected for 2 s (integration time) in the range of 1050-1700 cm$^{-1}$.

7.2.5. Data Analysis

The fluorescence images were analyzed using ImageJ software (National Institute of Health, Bethesda, MD). The fluorescence intensity profiles were plotted with Origin (OriginLab, Northampton, MA). The SERS spectra were calibrated and analyzed using GRAMS (Thermo Fisher Scientific Inc., Waltham, MA). The calibration curve from the optoelectrofluidic immunoassay of AFP was fitted and plotted with Origin software.
Figure 7.1. An experimental setup for optoelectrofluidic sandwich immunoassay (a) Schematic diagram and (b) pictures.
7.2.6. Procedure for Optoelectrofluidic Immunoassay

The image-driven immunoassay system based on the optoelectrofluidics is depicted in Figure 7.2(a). After the sample injection step, there are four steps, which were automatically controlled by an applied AC signal and a programmed LCD image: (1) global concentration of supporting microspheres and mixing of the samples; (2) washing of free analytes and probe nanoparticles; (3) local concentration of the immunocomplexes; and (4) the optical measurement of the amount of probes on the immunocomplexes. At first, the suspended microspheres were concentrated due to DEP and ACEO induced by a programmed LCD image in the application of the AC signal, of which frequency is ranged from 1 to 100 kHz. At this step, as a dark circle became smaller gradually for a certain time – 90 s in this experiment, the efficient mixing of sample solution as well as the concentration of microspheres also arose by the ACEO vortices. This optoelectrofluidic mixing effect facilitates rapid formation of immunocomplexes. Here the time and efficiency for immunoreactions is controllable by programming the LCD image. After the animated image was fully-processed and stopped in a form of a small dark circle, of which diameter is around 50 μm in this experiment, the AC frequency is changed into the range from 0.1 to 1 kHz for a few seconds – within 10 s in this experiment. In this washing step, free probe nanoparticles are washed out from the dark area by the strong ACEO vortices and immobilized onto the
photoconductive surface by the immobilization force, while the microspheres are concentrated into or still mixed with the other samples as moving along the ACEO vortices around the area due to the combination of ACEO, DEP and the gravity forces. When the applied AC frequency is changed into above 10 kHz, all the concentrated and washed immunocomplexes are concentrated and assembled into the dark area by the negative DEP forces and the electrostatic dipole interactions. Finally, an optical signal from the probe nanoparticles captured on the microspheres could be detected, after the applied voltage and the LCD image are turned off. Here a significant optical signal from the probe nanoparticles, which formed immunocomplexes, can be detected with large amount of antigens as shown in Figure 7.2(b). The detailed principles for the motion of particles in the optoelectrofluidic device are described in the Thesis and Simulation and Discussion.
Figure 7.2. Schematic illustration of (a) procedure for optoelectrofluidic immunoassays and (b) corresponding Raman spectra with and without target analytes.
7.3. Theory and Simulation

The electrokinetic mechanisms, which include DEP and ACEO, are main driving force for particle manipulation using the optoelectrofluidic device. In addition, we could also observe the electrostatic interactions due to the polarization of dielectric particles. The electrokinetics mechanisms act on the microspheres in concert depending on the frequency of the applied AC signal as presented in Chapter 3 and 4. For example, at the low-frequency range below 1 kHz, hydrodynamic drag force due to the strong ACEO flow becomes more dominant than other forces. Here, the DEP and the electrostatic dipole forces can also affect the behaviors of microspheres. At the high-frequency range above 10 kHz, the ACEO flow becomes much weaker; hence the DEP force becomes the most dominant force. Since most polymer microspheres show the negative DEP motion in low-conductivity media, they are repelled from the image patterns in the optoelectrofluidic device. On the other hand, the DEP force acting on the nano-sized particles is negligible because the magnitude of the DEP force is proportional to the volume of particles. However, the positive DEP force can attract the metallic nanoparticles, which were collected by ACEO flows and positioned near the surface, for immobilizing them onto the illuminated area [54].

The simulated results of the electric field distribution and the ACEO flows in the optoelectrofluidic device are shown in Figure 7.3. The ACEO
fluid velocity was simulated as a slip condition at the surface of the photoconductive layer. For the simulation, the Debye length was calculated using Gouy-Chapman equation as 55.7 nm. The voltage drop across each layer was calculated using an equivalent circuit model. When the AC voltage of 10 V_{pp} at 1 kHz was applied the voltage drop across the sample solution was calculated as 7.6 V_{pp} in the illuminated area. According to the simulation, the ACEO flows would occur toward the illuminated area along the surface of the photoconductive layer. By the viscous property of the fluids, the vortices would be induced in the sample solution around the edges of the image pattern. The direction and the relative magnitude of the negative DEP force acting on the microspheres were easily predictable based on the distribution of the electric field. The electric field was the strongest at the edge of the projected LCD image, and it was the weakest at the center of the dark area. Therefore, the negative DEP force repels microspheres from the image pattern to the dark area. Through this simulation study, we could estimated that the microspheres, which are supporting substrates for immunocomplexes, would be concentrated into the dark area by the combination of negative DEP, ACEO, and gravity when the LCD image pattern moves continuously. On the other hand, the probe nanoparticles and the antigens, which were not bound onto the microspheres, would be washed out by strong ACEO flows diverging from the dark area, where the immunocomplexes were concentrated.
Figure 7.3. Simulation results for the electric field distribution and the optically induced ACEO flows (blue arrows) in an optoelectrofluidic device. According to the LCD image pattern (red), the electric field distribution and the ACEO flow pattern are also changed.
7.4. Results

7.4.1. Optoelectrofluidic Concentration of Microspheres

The process of image-driven automated immunoassay is shown in Figure 7.4. A programmed LCD image, in which a dark circle becomes smaller for about 90 s, was utilized for our experiments. The behavior of microspheres showed good agreement with the estimation based on the numerical simulation shown in Figure 7.3. The microspheres were successfully concentrated by the animated LCD image in the application of 1 kHz frequency. After the LCD image was stopped in a form of 50 µm-diameter dark circle, some concentrated microspheres were still moved along the ACEO vortices and mixed with other samples. When we changed the AC frequency from 1 kHz into 10 kHz, all the concentrated microspheres assembled and formed chain-like structure by the electrostatic interactions. Finally, we could detect SERS from the immunocomplexes by projecting a laser spot after turning off the LCD image and the AC voltage. The time for processing all the steps took less than 5 min. Here, the washing of free antigens and probe nanoparticles is also simultaneously processed, but it could not be determined this general optical observation. So, we determined the washing effect due to the optoelectrofluidics with fluorescence nanoparticles.
Figure 7.4. Microscopic pictures of microspheres being processed for optoelectrofluidic immunoassay.
7.4.2. Determination of Washing Effect

To investigate the feasibility of the optoelectrofluidic sandwich immunoassay, a qualitative experiment was performed with 0.1 ng/mL rIgG-biotin as a target analyte. Here fluorescent (Max. Ex/Em: 441/486 nm) 6 µm-diameter PS microspheres conjugated with monoclonal anti-rIgG and neutravidin-coated fluorescent (Max. Ex/Em: 580/605 nm) 40 nm-diameter PS nanoparticles were used as supporting substrates and probe nanoparticles, respectively, as shown in Figure 7.5(a).

After the image-driven automated processes for the immunoassay of rIgG-biotin, we observed the fluorescence images of the processing area as shown in Figure 7.5(b). According to the microscopic pictures, the 6 µm-diameter supporting microspheres were successfully concentrated by the animated LCD image as shown in the left panel of Figure 7.5(b). In addition, the decrement of red fluorescence intensity within the microspheres-concentrated area was observed as shown in the right panel of Figure 7.5(b). This result might be due to the optoelectrofluidic washing of free fluorescent nanoparticles in the localized area.
Figure 7.5. (a) Schematic illustration of the formation of immunocomplex for fluorescence-based assay. Biotinylated rabbit IgG and neutravidin-coated fluorescent nanoparticles were applied for target analytes and probe nanoparticles, respectively. (b) Microscopic pictures after all steps for optoelectrofluidic immunoassay against the emission wavelength (left: green, right: red). The dotted circle is a dark area of the LCD image, which the microspheres are concentrated into and the nanoparticles are washed out from.
We also measured the intensity profile across the microspheres inside and outside the washed area. Within the washed area, red fluorescence was appeared only on the same position of concentrated microspheres in the washed area as shown in Figure 7.6(a). The intensity of fluorescence signal implies the amount of fluorescent nanoparticles, which formed sandwich immunocomplexes through the medium of rIgG-biotin molecules. While on the other, In the case of a microsphere, which was failed to concentrate and wash, the measurement of red fluorescence from the probe nanoparticles, which bound on a supporting microsphere, was impossible due to the interference by large amount of free probe nanoparticles around the microspheres as shown in Figure 7.6(b). These results ensure that the mixing, concentration, and washing steps for immunoassay can be conducted by the image-driven automated processes.
Figure 7.6. Fluorescence images and the intensity profiles of a microsphere (a) inside and (b) outside the washed area. Red solid line and green dotted line depict the fluorescence intensity profiles across the dotted line in the bottom (red) and the upper (green) images, respectively.
7.4.3. Quantitative Immunoassay of Alpha-Fetoprotein

The quantitative assay of human AFP based on SERS was performed using this optoelectrofluidic immunoassay platform. Here, monoclonal anti-AFP-conjugated PS microspheres were used as supporting material, and polyclonal anti-AFP-conjugated AgNPs, which are labeled with MGITC, were utilized as SERS probe nanoparticles as shown in Figure 7.7(a).

The SERS spectra of the sandwich immunocomplex against various concentrations of AFP are illustrated in Figure 7.7(b). The concentration of AFP was varied from 0.1 ng/mL to 1.5 ng/mL. The SERS signals were obviously increased as the concentration of AFP increased. The blank spectrum was obtained without the target antigen. The weak SERS signal in the absence of antigens implies that some MGITC-tagged AgNPs were still remained in the washed area or were nonspecifically bound onto the microspheres.

Among several prominent peaks in the spectrum of MGITC, the peak at 1615 cm\(^{-1}\) was chosen for the quantitative evaluation of AFP antigens, because the baseline of this peak (1555 – 1640 cm\(^{-1}\)) was flatter than others. The intensity of the peak was plotted as a function of the AFP concentration in Figure 8. The Raman peak intensity linearly increased, as the concentration of AFP increased from 0 to 1.5 ng/mL. The equation for the linear regression line was \(y = 3302x + 216.9\), and the correlation coefficient was 0.9910 (\(n = 6\)). The lower detection limit (LDL) of this
optoelectrofluidic SERS-based immunoassay for AFP was about 20 pg/mL, which is lower two orders of magnitude than that of the conventional ELISA methods. This optoelectrofluidic immunoassay platform required total sample volume of less than 500 nL, which is much less than that for ELISA. In addition, the time for one assay took less than 5 min, and manual handling of liquids was not required except for injecting samples into the slide glass-like OET device.

7.4.4. Simultaneous Multiple Experiments

Multiple assays could also be simultaneously performed by applying an LCD image, which generates a microarray composed of numerous virtual wells (dark area) for concentration and washing immunocomplexes as shown in Figure 7.9. This result shows that the multiplexed immunoassays could be conducted on the basis of concentrated microspheres in each well.
Figure 7.7. (a) Schematic illustration of the formation of immunocomplex for surface-enhanced Raman scattering (SERS)-based assay. Human alpha-fetoprotein (AFP) and malachite green isothiocyanate-tagged silver nanoparticles were applied for target analytes and probe nanoparticles, respectively. (b) SERS spectra for various concentrations of AFP antigen.
Figure 7.8. Intensity of the SERS signal at 1615 cm⁻¹ against the concentration of alpha-fetoprotein. A linear relationship is shown in the concentration range from 0 to 1.5 ng/mL. The linear fitting line is $y = 3302x + 216.9$, and the correlation coefficient was 0.9910 ($n = 6$).
Figure 7.9. (a) Single fluorescent microsphere array (5×5) for determining the washing effect in microarray. (b) 3×3 microarray of concentrated microspheres for simultaneous multiple immunoassays based on surface-enhanced Raman scattering.
7.5. Discussion

7.5.1. Influence of Aggregation of Nanoparticles

According to the calibration curve shown in Figure 7.8, not only the value of peak intensity, but also the standard deviation increased as the antigen concentration increased over 1 ng/mL. When the AFP concentration was over 2 ng/mL, the reproducibility of SERS signal was significantly decreased. These phenomena might be due to the aggregation of MGITC-tagged AgNPs by AFP solution. We could optically determine the silver aggregates, of which size is over several hundreds of nanometer, as soon as we injected the 2 ng/mL AFP solution. The large silver aggregates were also concentrated into the dark area and were not washed completely. However, this matter is not restricted to the case of this optoelectrofluidic immunoassay platform. In practice, most of the SERS-based biosensors have such the problems due to the aggregation of metal nanoparticles. If the limitation of SERS technology can be overcome, broader dynamic range of the immunoassay would be obtained.

7.5.2. Causes of Blank Signal

Even in the absence of AFP, a weak SERS signal was observed in Figure 7.7(b). There are two reasons: (1) some probe nanoparticles still remained in the washed area; (2) nonspecific binding of antigens or probe
nanoparticles onto the microspheres. The former might be due to the behavior of nanoparticles by optoelectrofluidic flows and thermal motion. Most of the nanoparticles were washed out from the dark image and immobilized into the illuminated area. According to the simulation depicted in Figure 7.3, however, there was still weak flow converging into the dark area on the bare ITO surface. The flow is originated from the main ACEO flow toward the illuminated area along the photoconductive surface. Although the strong EHD flow washed out the free nanoparticles and molecules, little influx of probe nanoparticles might be slowly occurred. The thermal motion of the washed nanoparticles after turning off the voltage at the detection step can also affect the background signal. However, according to our experiments with fluorescent nanoparticles and a previous literature [54], the dispersion of washed or patterned nanoparticles due to their Brownian motion was negligible. The latter, which is relative to the nonspecific absorption of antigens and nanoparticles, may be caused by electrostatic forces as well as by chemical binding forces. In-depth consideration for the electrostatic particle-particle interactions in this optoelectrofluidic immunoassay was discussed as follows.
7.5.3. Electrostatic Particle-Particle Interactions

At the AC frequencies ranged from 1 kHz to 100 kHz, which include our experimental conditions, the electrostatic particle-particle interactions due to the induced-dipole of microspheres were observed. The attractive force, which aligns the microspheres in the direction of electric field, became stronger at 10 kHz than at 1 kHz, resulting in the ‘perl chains’ of concentrated microspheres. This chain structure of immunocomplexes helped the highly-sensitive detection of antigens by increasing the amount of probe nanoparticles exposed to the focal volume of the incident laser. If the microspheres are assembled in a monolayer, the SERS from only one immunocomplex will be detected. However, in this experimental condition, at least three to five 6 μm-diameter microspheres form a chain, of which length is enough to cover the focal volume (~ 10^{-17} m^3) of the incident laser, in the direction of the beam. In addition, the contact points among the microspheres in a chain can serve as hot spots for enhancing SERS signal from the probe nanoparticles. However, this chain structure may interfere with the reproducible SERS detection. The chain began to relax as soon as turning off the applied voltage for SERS detection. As a consequence, the SERS signal gradually reduced with a large fluctuation from about 30 s after the AC signal was intercepted. Therefore, we could obtain reproducible SERS intensity for only 30 s after the voltage is turned off. The electrostatic interactions may also induce nonspecific binding of probe
nanoparticles onto the microspheres. However, according to our experiments with fluorescence, the electrostatic attraction between microspheres and nanoparticles was observable only at extremely low-frequency range around 100 Hz. Therefore, the electrostatic binding of nanoparticles onto microspheres would be negligible in our experimental conditions ranged from 1 kHz to 10 kHz. However, it is difficult to conclude that there is no nonspecific binding of probe nanoparticles by electrostatic interactions yet, thus this matter should be investigated and solved for lower detection limit.
7.5.4. Influence of Photoconductive Layer Position

The position of photoconductive layer in the device also affected the movement of microspheres. We have already investigated the effect of gravity on the DEP-based manipulation of large and heavy oocytes [36]. Even in this study, in which 6 μm-diameter PS microspheres were manipulated with an EHD mechanism, the gravity force seriously influenced the particle behaviors. According to the previous results, when the photoconductive layer was at the bottom as the conventional setup for optoelectrofluidic platform, both the microspheres and the nanoparticles suspended around the projected image pattern were rapidly moved to the illuminated area by the optically induced ACEO flow and positive DEP force at 1 kHz [54, 61, 64]. However, in our experiments, wherein the photoconductive layer was at the upper of the device, the microspheres were repelled from the image pattern, while the nanoparticles were concentrated into the area at the same frequency condition.

These phenomena are interpretable based on the force balance among the hydrodynamic drag force by ACEO, the DEP force, and the gravity force as shown in Figure 7.10. We assumed that an ac voltage with 1 kHz frequency was applied and the extremely low-conductivity media was used as a sample buffer like our experimental condition. In such the low-frequency, both the microspheres and the nanoparticles experience strong ACEO and are rapidly dragged towards the center of the light pattern. The
polymer microspheres also experience the negative DEP and the gravity forces. As a consequence, the net force concentrates both the microspheres and the nanoparticles into the illuminated area in the conventional upright mode, wherein the photoconductive layer is at the upper of the device. In the inverted mode, however, the microspheres are repelled from the light pattern. This might be due to the effect of the negative DEP and the gravity forces, which push out the microspheres from the surface of the photoconductive layer in the inverted mode of the device. In the case of the nanoparticles, they only experience the hydrodynamic drag force by ACEO, thus they are concentrated into the illuminated area in both modes. The weak positive DEP force can also help the immobilization of the metallic nanoparticles onto the illuminated area of the photoconductive surface [54].

By using the inverted mode of the optoelectrofluidic device, we could selectively concentrate immunocomplexes as washing out free probe nanoparticles. This is a new finding about the physical mechanisms for selective concentration of micro- and nanoparticles using an optoelectrofluidic device. If more complete model for simulating these phenomena is formulated on the basis of more experimental investigations, it would play an important role in improvements of this optoelectrofluidic particle separation and its applications.
Figure 7.10. Illustration for forces acting on the microspheres and behaviors of microspheres and nanoparticles in conventional upright mode (upper) and inverted mode (bottom) of the optoelectrofluidic device.
7.6. Summary

In this Chapter, an optoelectrofluidic immunoassay platform has been developed for simple, automated, fast (~ 5 min), and highly-sensitive (LDL = 20 pg/mL) detection of AFP in tiny sample volume (~ 500 nL) using SERS. This image-driven immunoassay using a conventional OET device has five significant meanings in several perspectives. First, this thesis described the first application for detecting biological molecules using an optoelectrofluidic device. Up to now, all the studies about optoelectrofluidics have been focused only to the manipulation of some objects, except only one study about applying an OET device for the measurement of molecular diffusion coefficient (See Chapter 6). In such the context, the optoelectrofluidic immunoassay has a significant meaning in respect that this is the first realization of the detection technology based on optoelectrofluidics. Second, much less volume of samples was required for the assay than the conventional ELISA systems. Only a several hundreds of nanoliter sample droplet, which contains supporting microspheres, target analytes, and probe nanoparticles, was dropped on the OET device for the immunoassay in this system. Third, any complicated robotic or fluidic components were not required for the automated immunoassays. Only an OET device, which looks like a slide glass, and an LCD module for controlling fluids were required. This simple structure and cheap components make it easy to commercialize and apply this technology for
clinical diagnosis and biochemical analysis. Fourth, by using the optoelectrofluidic immunoassay, it took much less time (~5 min) to conduct all the processes for sandwich immunoassay than conventional methods. The incubation time shortened due to the enhanced mixing effect by the optically induced electrohydrodynamic vortices, as well as the fully-automated processes without pipetting liquid samples allows us to save times. Finally, the image-driven manipulation platform allows more flexible uses of this technology. For example, parallel multiple assays were possible based on an LCD image programmed for generating multiple areas for concentration, reaction, washing, and detection. Based on this approach, a new multiplexed immunoassay platform could be constructed using an array of optically controlled virtual microwells. This optoelectrofluidic sandwich immunoassay will provide a new way for simple, fast, automated, and highly-sensitive detection of antigens.
PART IV

CONCLUSIONS AND PERSPECTIVES
Part IV presents conclusions and perspectives. Research summary and conclusions are presented in Chapter 8. This Chapter summarizes what I have done during my Ph.D. course and provides answers for some questions; “Where is the optoelectrofluidics really useful?” and “How can it be applicable to chemistry and biology?” Challenges and future works, which should be overcome and established for growth of the optoelectrofluidic technology in chemical and biological fields, are also presented in Chapter 9. In detail, a scheme for multiplexed optoelectrofluidic immunoassays as an extended type of the scheme presented in Chapter 7 is suggested. Optoelectrofluidic platforms for reproducible, highly-sensitive, and tunable SERS detection using optoelectrofluidic concentration and assembly of metal nano/microparticles are also presented. In addition, a method for quantify proteins in immunocomplexes based on the change of their optoelectrofluidic mobility is presented with a preliminary data.
Chapter 8: Conclusions

Here, we presented manipulation and dynamic control of particles and molecules using an optoelectrofluidic manipulation platform such as OET. Firstly, separation and concentration of microparticles has been performed using lab-on-a-display, which is an LCD-based optoelectrofluidic platform. In this study, 1 μm-diameter PS particles were completely separated from the mixture of 1 μm and 6 μm particles within 30 s with application of a voltage of 10 V_{pp} at 10 kHz, and spontaneously concentrated. We also investigate the frequency-dependent behavior of 1 μm and 6 μm-diameter PS particles in an optoelectrofluidic device at the AC frequencies ranged from 100 Hz to 10 kHz. Secondly, dynamic control of colloidal assembly has been performed using a projector-based optoelectrofluidic platform. We simulated main forces, such as DEP and ACEO drag, for optoelectrofluidic particle concentration, and investigated colloidal assembly based on the electric-field-induced flows, such as ICEO and FCEO, and electrostatic particle-particle interactions in an optoelectrofluidic device. Optoelectrofluidics allowed the control of both the assembly pattern over a large area and the distances between the assembled particles by modulating the projected light pattern and the applied voltage. Thirdly, dynamic control of local chemical concentration in a molecular solution has been
demonstrated using OFM, which an optoelectrofluidic device is integrated into a conventional fluorescence microscope. We investigated the optoelectrofluidic change of local chemical concentration against the applied AC frequency, the type of molecules, and the bulk concentration. The investigation of dipole-dipole attraction among the molecules in the optoelectrofluidic device was also performed. Temporal and spatial control of local molecular concentration has been demonstrated.

On the basis of the basic studies about the optoelectrofluidic manipulation of micro-/nanoparticles and molecules, new schemes for measuring or detecting biomolecules using optoelectrofluidics have been developed. First of all, a simple method for measuring diffusion coefficient of molecules in a fluid based on an optoelectrofluidic device has been demonstrated. The diffusion coefficients of various FITC-dextran molecules in solution could be measured using the fluorescence recovery after the optoelectrofluidic local molecular depletion. The diffusion coefficients of 10, 40, and 500 kDa FITC-dextran measured using the OFM were $125.1 \pm 7.1$, $46.1 \pm 2.9$, and $22.5 \pm 1.7 \times 10^{-8}$ cm$^2$/s, respectively. These experimental values were significant to the previously reported values measured by FRAP techniques and the theoretical values calculated from the Stokes-Einstein equation. Next, image-driven sandwich immunoassays using optoelectrofluidics has been developed for simple, rapid, automated, and highly-sensitive detection of antigens. This
optoelectrofluidic immunoassay platform requires very tiny volume of samples below 500 nL and very short time within 5 min for the assay. In addition, any complicated robotic or fluidic components were not required for the automated immunoassays. The LDL for human tumor marker, AFP, was around 20 pg/mL, which is lower two orders of magnitude than that of the conventional ELISA methods. Parallel multiple assays were also possible based on an LCD image programmed for generating multiple areas for concentration, reaction, washing, and detection.

Although many types of OET platform has been developed and many studies for programmable manipulation of various target objects using it has been reported until now, none of the research could show the practical application of the optoelectrofluidic manipulation in biological and chemical fields. In other words, most of the researchers have mentioned that the optoelectrofluidic technology can manipulate various materials and this manipulation technique can be applied various applications, but have never demonstrated where this elegant manipulation technique is really useful in practice. In this point of view, it is noted that this thesis has a significant meaning in respect that it presents the first practical biological applications, which include measurement and detection of biomolecules, of the optoelectrofluidic manipulation technology.
Chapter 9: Challenges and Future Works

9.1. Challenges

Despite fascinating capability of the optoelectrofluidic device, some challenges remain.

First, the performance of this optoelectrofluidic device is basically dependent on the conductivity of media. For a salty media such as blood plasma or cell culture media, the photoconductivity should be much higher than the conventional OET device based on a-Si:H. The phototransistor-based OET device could be utilized for increasing the photoconductivity of the device and applying this technique for high-conductivity physiological buffers [47].

Second, the performance depends on the applied AC frequency as well. A process for optimization of the AC frequency condition, therefore, is always required before applying different target samples. In Chapter 7, for example, the silver nanoparticles were washed well and immobilized onto the illuminated area at higher frequency above 1 kHz, while relatively low ac frequency from 100 Hz to 1 kHz was optimized condition for the fluorescent polymer nanoparticles. For compensating these limitations, more theoretical studies for clarifying the theoretical rationale of behavior of objects in the optoelectrofluidic device should followed.
Third, thick glass substrate and opaque photoconductive layer can interfere with the effective measurement of optical signals as well as flexible modification of optical pathway. This limitation could be overcome by developing a new device, by thinning the substrate, or by applying sensitive probes for more flexible and sensitive optical detection. Devices integrated with optical fibers or microlens arrays would also be useful for portable applications.

Finally, the integration of microfluidic components for manipulating multiple sample solutions would be very helpful for conducting more complicated processes. Such the research for integrating microfluidic channels [48] or an electrowetting device [134] into the optoelectrofluidic device has already been performed.
9.2. Future Works

9.2.1. Multiplexed Optoelectrofluidic Sandwich Immunoassays

The schematic diagram for the microarray-based approach for multiplexed immunoassays is shown in Figure 8.1. In Chapter 7, the possibility of multiplexed immunoassays using the optoelectrofluidic device has been depicted with some experimental results using an LCD image, which forms a microarray (See Figure 7.9). The optoelectrofluidic technology has several advantages that tiny sample volume within several hundreds of nanoliter is required and no complicated electrical and fluidic components are required for high-throughput process. Therefore, it is possible to inject multiple liquid samples into a device by patterning hydrophilic/hydrophobic area or by integrating microwells and to simultaneously perform multiple immunoassays using a programmed LCD image. By patterning antibody array onto the ground electrode, which is coated with a transparent conductive material such as ITO and disposable, numerous experiments for detecting multiple target analytes, which are complementary to the surface-patterned antibodies, would be possible at the same time. Quantification would be possible by applying various probes such as quantum dots or SERS probe nanoparticles. In the case of fluorescence, integration of an optoelectrofluidic device into a confocal microscopy could be utilized for reproducible and accurate quantification.
Figure 9.1. Schematic diagram of microarray-based approach for multiplexed immunoassays.
9.2.2. Optoelectrofluidic Surface-Enhanced Raman Scattering Platform

Here a method for \textit{in situ} measurement of SERS enhanced by optoelectrofluidic local concentration of gold nanoparticles (AuNPs) or gold-coated microspheres is presented. When we apply an AC voltage to an optoelectrofluidic device and project a light into its photoconductive surface, resistance of the partially illuminated area decreases, resulting in a nonuniform electric field. In the optically induced nonuniform electric field, several mechanisms including DEP, ACEO, and electrostatic interaction forces make the particles and target molecules to be concentrated into the illuminated area. By adjusting the applied voltage, the roughness and assembly morphology of the concentrated AuNPs or gold-coated microspheres can be controlled, resulting in a dynamic tunable SERS substrate.

In the case of AuNPs, only one light source is required, because the size of laser spot, which is for SERS detection, is large enough to concentrate and assemble AuNPs into a certain area. We have already demonstrated the optoelectrofluidic enhancement of SERS from rhodamine 6G (R6G) molecules by concentrating AuNPs in the optoelectrofluidic platform as shown in Figure 8.2(a). In the application of an AC voltage and a laser into an optoelectrofluidic device, AuNPs are concentrated and assembled within the illuminated area by optically induced electrokinetic and electrostatic mechanisms, resulting in a SERS substrate. In this
experiment, 633 nm He/Ne laser source (2.5 mW) was utilized for inducing an electric field in a sample solution, which is located in an optoelectrofluidic device. After the application of an AC voltage to the optoelectrofluidic device, SERS signal from the sample was transmitted into a spectrometer through a beam splitter and a Raman filter. Integration time was fixed as 1 s. SERS spectra of 50 μM R6G with AuNPs in an optoelectrofluidic device is shown in Figure 8.2(b). In the case of R6G, the SERS signal increased according to the time for 2 min in the application of the voltage of 10 V_{pp} at 100 kHz. At 100 kHz, the fluorescence signal from R6G was not changed, and decreased at the lower AC frequency ranged from 100 Hz to 10 kHz, as like the case of tiny fluorophore molecules, which have been studied in Chapter 5. However, the AuNPs-based SERS signal was slightly increased even at the low frequency range. This increment might be due to the concentration and assembly of AuNPs by the laser-induced electrokinetic mechanisms.
Figure 9.2. (a) Schematic diagram of optoelectrofluidic enhancement of surface-enhanced Raman scattering (SERS). (b) As time goes by, after applying a voltage and projecting a laser source, the SERS signal from rhodamine 6G was increased due to the optoelectrofluidic concentration and assembly of gold nanoparticles.
One more concept based on gold-coated microspheres has also been demonstrated with preliminary experiments. As shown in Figure 8.3 (a), we applied two light sources; one for optoelectrofluidic patterning of gold-coated microspheres and the other for SERS detection. Here 4,4′-dipyridyl (DP) molecules were adsorbed onto the gold-coated microspheres. The Raman scattering signal of DP molecules enhanced by the surface of gold-coated microspheres were detected as shown in Figure 8.3(b). In the crystalline structure of the gold-coated microspheres assembly, the maximum SERS intensity was detected at the gap of two microspheres (A). This result might be due to the formation of hot spot between two microspheres, which are almost in contact with each other. In the same context, the SERS intensity at the gap among three microspheres (B) has the value smaller than that at the gap between two microspheres (A) and larger than that at the center of one microsphere (C). The gap between (among) the microspheres is tunable according to the study about optoelectrofluidic colloidal assembly (See Chapter 4). Therefore, this approach will provide an easy and simple way to prepare reproducible and reconfigurable SERS-active substrate.
Figure 9.3. (a) Schematic diagram of surface-enhanced Raman scattering (SERS) platform based on the optoelectrofluidic colloidal assembly. (b) The SERS spectra of 4,4'-dipyridyl shows different values against the position of laser spot on a colloidal crystal formed in an optoelectrofluidic device.
9.2.3. Optoelectrofluidic Immunoassays Using Light Scanning Method

According to our experiments for the optoelectrofluidic sandwich immunoassays, the velocity of concentrating immunocomplexes depended on the amount of target analyte and probe nanoparticles attached onto the microspheres. This result might be due to the change of dielectric property of the microspheres by the attachment of nanoparticles or molecules. On the basis of this phenomenon, we designed a new method to quantify the amount of target analyte in a solution without optical detection methods such as fluorescence and SERS. Here we measure the maximum velocity of immunocomplexes, which is composed of supporting microspheres, human immunoglobulin G (hIgG) as the target analyte, and gold nanoparticles for inducing change of surface conductivity as shown in Figure 8.4(a). According to the preliminary data, which is shown in Figure 8.4(b), the moving velocity of the microspheres by optically induced DEP was around – 0.7 μm/s and 1 μm/s, when the hIgG concentration was 0 and 100 ng/mL, respectively. The AC frequencies ranged from 500 kHz to 1 MHz are the conditions at which only the DEP force affect the behavior of microspheres. Therefore, this result means that the behavior of plain microspheres, which originally shows negative DEP, was changed into positive DEP by the gold nanoparticles, which were attached onto the microsphere surface through the medium of hIgG molecules. The DEP velocity change would be dependent on the amount of the attached gold nanoparticles, thus it would
be changed according to the amount of the target molecules. By measuring the DEP velocity of microspheres using an optoelectrofluidic device, therefore, the amount of target molecules could be indirectly quantified. Here a simple method based on light-scanning can be applied for more simple measurement system without real time image processing for velocity measurement. We can program a scanning light pattern, of which scanning velocity decreases as passing through every step. The number of particles, which is trapped by the light pattern, would be increased as the lowering scan rate. By finding a specific step (scanning velocity), at which half of all the microspheres were successfully trapped and moved, we can define a value for quantifying the amount of target analytes. This approach based on the optoelectrofluidic mobility of immunocomplexes will provide very simple and easy way to quantify the antibody-antigen interactions without complicated optical and fluidic components, in tiny sample volume around several hundreds of nanoliter.
Figure 9.4. (a) Schematic diagram of optoelectrofluidic immunoassay platform based on a light scanning method. (b) The optoelectrofluidic mobility of immunocomplex microbeads according to the analyte concentration.
# Appendix A:
## Fabrication Processes for Optoelectrofluidic Device

### 1) Photoconductive layer

Starting material: 6 × 6 inch² ITO-coated glass substrate (purchased from Samsung–Corning Precision Glass, Asan, Korea)

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Machine</th>
<th>Parameter</th>
</tr>
</thead>
</table>
| 1     | n⁺ a-Si:H deposition | PECVD | 1.5% PH₃ in SiH₄  
 20% SiH₄/He = 300 sccm  
 H₂ = 100 sccm at 280°C |
| 2     | Intrinsic a-Si:H deposition |  | 20% SiH₄/He = 300 sccm  
 H₂ = 100 sccm at 280°C |
| 3     | SiNx deposition |  | SiH₄, NH₃, N₂ mixture |
| 4     | SU8 spin | Spin coater | SU8 3025 (25 μm thickness)  
 10 s at 500 rpm  
 30 s at 2000 rpm |
| 5     | Soft bake | Oven | 1 min at 65°C  
 10 min at 95°C |
| 6     | UV exposure | Aligner | 10 s at 20.3 mW/cm² |
| 7     | Post-exposure bake | Oven | 1 min at 65°C  
 3 min at 95°C |
| 8     | Develop | Hood | 5 min in SU8 developer  
 Dip in IPA  
 Rinse with water |
| 9     | Opening some region of ITO for bias connection | RIE | 10% O₂  
 90% SF₆  
 100 W RF power |
| 10    | PR removal | Hood | 10 min in 50°C acetone  
 Dip 5 min in methanol  
 Rinse with water |
| 11    | Dicing devices | Dicing saw | 37.5 × 25.0 mm² |
2) **Ground layer with PR spacers**

Starting material: 4 inch-diameter ITO-coated glass substrate
(purchased from Samsung−Corning Precision Glass, Asan, Korea)

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Machine</th>
<th>Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dehydration</td>
<td>Oven</td>
<td>15 min at 200°C</td>
</tr>
<tr>
<td>2</td>
<td>THB-151N spin</td>
<td>Spin coater</td>
<td>THB-151N (30 μm thickness)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20 s at 300 rpm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30 s at 1750 rpm</td>
</tr>
<tr>
<td>3</td>
<td>Soft bake</td>
<td>Oven</td>
<td>3 min at 120°C</td>
</tr>
<tr>
<td>4</td>
<td>Stabilization</td>
<td>Table</td>
<td>5 min</td>
</tr>
<tr>
<td>5</td>
<td>UV exposure</td>
<td>Aligner</td>
<td>60 s at 9.7 mW/cm²</td>
</tr>
<tr>
<td>6</td>
<td>Develop</td>
<td>Hood</td>
<td>3 min in THB-151N developer</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dip in IPA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Rinse with water</td>
</tr>
<tr>
<td>7</td>
<td>Dicing devices</td>
<td>Dicing saw</td>
<td>37.5 × 25.0 mm²</td>
</tr>
</tbody>
</table>
본 학위논문에서는 광전자유체제어 기술을 이용한 생체분자의 측정과 검출에 관한 연구를 수행하였다. 광전자유체제어 기술이란, 광학적인 방법을 통해 전기동역학적 힘을 유도하여 미세입자나 유체의 운동을 제어할 수 있는 기술이다. 1995년에 전기장에 적외선 레이저를 조사하여 유체의 온도가 국부적으로 상승하도록 함으로써 전열효과에 의한 유동이 발생하게 한 바 있으며, 2000년에는 전압이 인가된 전극에 특정한 패턴의 자외선을 조사하여 전하를 지닌 미세입자가 전기영동에 의해 전류밀도가 높은 방향, 즉 자외선이 조사된 방향으로 이동시키는 방법이 보고된 바 있다. 2005년 발표된 광전자잡게 기술은 전극 상에 비정질 실리콘과 같은 우수한 광전도성 물질을 증착시킨 형태로써, DMD와 같은 디스플레이 장치를 거친 백색광 패턴을 광전도성층에 조사하면, 빛이 조사된 영역에만 전류가 도통하여 가상의 전극이 형성되는 원리를 이용한 광전자유체제어 기술이다. 이러한 광전자잡게 기술은 움직이는 영상으로 세포와 같은 미세입자들의 유전영동을 유도하고, 자유자재로 구동할 수 있도록 한다. 이러한 광전자잡게 기술은 특정한 형태의 전극을 형성시키기 위한 고정된 전극 패턴을 복잡한 공정과정을 통해 제작할 필요가 없고, 빛의 패턴만을 바꾸어 줄으로써 다양한 형태의 전극을 자유롭게 형성시키고 제어할 수 있다. 또한, 단순한 평판 구조를 지닌 소자의 특성상 저비용 대량생산에 용이하고, 대면적, 대용량 프로세스, 휴대
용 장치를 위한 집적화와 저전력 구동에 유리하다. 뿐만 아니라, 값비싼 레이저 광원이나 복잡한 광학 부품이 요구되는 기존의 광학 집계와 달리, 광학적으로 유도된 전기동역학적 원리를 사용하기 때문에, 10만배 이상 약한 광원에 의해서도 미세입자의 조작이 가능하다는 장점이 있다.

본 연구에서는 광전자집계 기반의 광전자유제제어 기술을 이용하여 생체분자의 측정과 검출에 관한 연구를 수행하였다. 이에 앞서, 교류 전기삼투 및 유전영동과 같은 다양한 전기동역학적 원리에 대한 이해를 위해 간단한 등가회로모델을 바탕으로 실험적으로 얻은 자료와 함께 이론적인 기초 연구를 수행하였다. 또한 LCD 기반 시스템과 형광 현미경 기반 시스템을 이용하여, 고분자성 미세입자와 생체분자의 광전자유체역학적 거동 특성을 조사하였으며, 미세입자의 빠른 농축과 분리, 조립, 생체 분자의 능도 조절 기술 등을 개발하였다.

미세입자의 농축과 분리를 위해서는, LCD 영상에 의해 유도되는 교류전기삼투 유동과 음의 유전영동을 이용하였다. 광전자유제제어 소자에 10 kHz 주파수의 교류전압을 인가하여, 1 µm 지름의 폴리스테린 입자를 1 µm와 6 µm 입자가 섞여있는 혼합물로부터 30초 내에 빠른 속도로 분리해내고, 이와 동시에 농축하는데 성공하였다. 이와 같은 결과는 입자의 크기와 교류전압의 주파수에 의존하는 전기동역학적 원리의 특성에 의한 것으로, 100 Hz 부터 10 kHz 범위에서 미세입자의 거동에 대한 이론적 연구도 수행하였다.
미세입자 조립의 광전자적 조절은 프로젝터 기반의 광전자유체제어 시스템을 이용하여 이루어졌다. 3 μm 폴리스티렌 입자를 사용하였으며, 1 kHz 이하의 주파수 영역에서 입자의 조립에 영향을 미치는 교류삼투 유동, 정전기적 상호작용 등에 관한 연구를 수행하였다. 광전자유체제어 소자에 조사된 빛 패턴을 조절함으로써, 미세입자 패턴을 조절하고, 이와 동시에 인가전압의 주파수를 조절함으로써, 미세입자 패턴의 내부 조립 특성, 즉 미세입자간 거리를 자유자재로 조절할 수 있었다.

마지막으로, 형광현미경에 집적된 광전자유체제어 소자를 이용하여, 하나의 광원만으로 유체 내 국소적인 영역에서의 생체분자 농도를 조절하고, 이와 동시에 검출할 수 있도록 하였다. 이 때 빛에 의해 유도된 교류삼투유동 및 정전기적 상호작용에 의해 형광 관찰을 위한 입사광 영역에서의 생체분자의 농도가 시공간적으로 조절됨을 확인하였으며, 이러한 현상은 분자의 종류, 인가 전압의 크기와 주파수, 빛 패턴 등에 의존하는 것을 실험적으로 확인하였다.

본 연구에서는 앞서 수행한 미세입자 및 생체분자의 조작에 관한 연구들을 바탕으로, 광전자유체제어 기술을 기반으로 한 생체분자의 측정 및 검출과 관련된 실질적인 응용 기술을 개발하였다. 광전자유체제어 기술은 오랫동안 세계적으로 많은 주목을 받아왔으나, 그에 관한 연구 경향은 나노선, 나노튜브, 세포, DNA 등 다양한 물질들을 조작하고 패턴화하는데 집중되어 왔다. 그러나 이러한 조작 기술을 바탕으로 실
질적인 생화학적 응용 기술은 아직까지 개발된 바 없다.

본 연구에서는 광전자유체제어 소자 내에서의 생체분자의 기동 특성을 이용하여 생체분자의 확산계수를 쉽고 빠르게 측정할 수 있는 기술을 개발하였다. 먼저 100 Hz 주변의 저주파 교류전압이 인가된 상태에서 빛이 조사된 영역으로부터 빠른 속도로 퍼지게 되는 원리를 이용하여, 분자가 존재하지 않는 고갈 영역을 형성시켰다. 이 후 전기장을 제거하여 퍼졌던 분자들이 확산을 통해 다시 고갈 영역에 채워지는 현상을 관찰한 후 100, 400, 500 kDa의 형광 덱스트란 분자의 확산계수를 측정할 수 있었다. 형광현미경을 이용하여 분자의 농도 변화를 실시간으로 관찰하였으며, 빛이 조사되는 영역을 조리개를 이용하여 조절함으로써 측정의 정밀도를 향상시킬 수 있었다. 측정된 확산계수는 각각 125.1 ± 7.1, 46.1 ± 2.9, and 22.5 ± 1.7 × 10⁻⁸ cm²/s 였으며, 이는 기존의 형광 광표백 기법을 이용해 측정된 값들과 매우 잘 일치하였다. 이러한 광전자유체제어 기술을 이용한 분자 확산 계수 측정방법은 기존의 확산계수 측정 방법과 달리, 강한 레이저 광원이나 초고속 카메라, 광표백 현상, 유체 구동을 위한 요소들을 요구하지 않으며, 빛 패턴 조절을 통해 측정의 정밀도를 조절할 수 있다는 장점이 있다.

또한, 광전자유체제어 기술을 기반으로 한 영상 기반 샌드위치 면역분석법을 개발하여, 수백 nL에 불과한 미세 유체 방울 내에서 암 표지 물질을 빠르게 고감도로 검출하는 데 성공하였다. 프로그램화된 LCD 영상으로 미세입자와 분자, 나노입자의 농축, 혼합, 분리를 제어함으로
써 자동화된 면역분석법을 수행할 수 있었다. 지지층으로 쓰이는 미세 입자들은 유전영동, 교류전기삼투, 중력 등의 영향으로 움직이는 LCD 영상으로부터 멀어져서 농축된다. 반면 형광표지 나노입자 또는 SERS 표지 나노입자의 경우에는 교류전기삼투 유동에 의해, 농축된 미세입자들로부터 분리된다. 이러한 현상을 이용하여 암 표지물질인 AFP를 매개로 하여 미세입자와 달라붙은 SERS 표지온 나노입자의 경우에는 세척이 되지 않고, 달라붙지 않은 나노입자들만 세척을 함으로써, 상기 미세 입자에 붙은 나노입자의 양을 SERS 신호를 통해 정확하게 측정할 수 있다. 이러한 방법을 적용하여 0 ng/mL 부터 1.5 ng/mL 까지 존재하는 AFP의 양을 정량적으로 측정할 수 있었으며 검출한계는 20 pg/mL로 측정되었다. 또한 500 mL 이하의 미세유체방울 내에서 5분 이내에 결과를 얻어낼 수 있었으며, 프로그램화된 LCD 영상을 이용하여 여러 번의 실험을 한 번에 자동으로 수행할 수도 있었다. 본 기술은 SERS 뿐만 아니라 형광 등 다양한 면역분석법에 적용할 수 있으며, 간단하게 적은 양의 샘플만으로 자동화된 면역분석을 수행할 수 있다는 장점이 있다.

마지막으로, 광전자유체제어 기술의 생물학적 응용을 위하여 해결되어야 할 과제 및 전망을 제안하였다. 광전자유체제어 기술은 복잡한 전극 구조와 전기구동 시스템, 미세체널이나 펌프 등의 유체조작 시스템 없이도 마당의 유체 샘플 내에서 빛을 이용하여 자유자재로 미세입자를 구동할 수 있는 기술이다. 또한 본 연구에서는 이러한 미세조작
기술을 바탕으로 생체분자의 측정 및 검출을 위한 새로운 응용기술을 제안하였다. 하지만, 광전자유체제어 기술은 근본적으로 전기동역학적 원리를 사용하기 때문에, 기술의 성능이 샘플의 전기적 특성과 인가된 전압신호의 주파수에 매우 의존한다는 특성이 있다. 따라서 더 높은 광 전도성을 지닌 새로운 물질 또는 새로운 소자의 개발이 요구되며, 전기 공학적, 유체역학적, 전기동역학적인 이론에 대한 깊은 고찰이 요구된다. 또한 단순한 소자의 구성은 이용상의 편리성과 제조단가, 유연성, 휴대성 등에는 매우 유리하나, 두 종류 이상의 유체를 다루는 복잡한 과정의 실험을 수행할 때에는 매우 불리하다. 따라서 미세유체 조작을 위한 소자의 집적화가 요구되며, 이러한 연구는 이미 활발히 진행 중이다.

본 기술을 바탕으로 항원의 다중동시검출을 수행하기 위하여, 개선된 광전자유체제어 면역분석 기술을 개발할 예정이며, 금속 나노입자 및 미세입자를 패턴화하여 SERS 검출을 위한 능동 소자를 개발하고자 한다. 또한 광학적 검출의 한계를 뛰어넘기 위해, 특이 물질 결합으로 인한 미세입자의 운동성 변화를 통해 단백질간 상호작용을 정량화할 수 있는 새로운 검출기술을 개발하고자 한다. 광전자유체제어 기술이 단순한 조작 기술로써의 기능을 뛰어넘어, 실용적인 응용 기술로 발전되기 위해서는 생체분자의 검출이나 측정과 같은 기술의 개발이 필수적으로 요구된다. 따라서, 본 연구를 통해 광전자유체제어 기술의 응용 분야가 더욱 확대되고, 기술적 가치가 크게 향상될 것으로 기대된다.
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