Lab on a Chip

Accepted Manuscript

This is an *Accepted Manuscript*, which has been through the RSC Publishing peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, which is prior to technical editing, formatting and proof reading. This free service from RSC Publishing allows authors to make their results available to the community, in citable form, before publication of the edited article. This *Accepted Manuscript* will be replaced by the edited and formatted *Advance Article* as soon as this is available.

To cite this manuscript please use its permanent Digital Object Identifier (DOI®), which is identical for all formats of publication.

More information about *Accepted Manuscripts* can be found in the **Information for Authors**.

Please note that technical editing may introduce minor changes to the text and/or graphics contained in the manuscript submitted by the author(s) which may alter content, and that the standard **Terms & Conditions** and the **ethical guidelines** that apply to the journal are still applicable. In no event shall the RSC be held responsible for any errors or omissions in these *Accepted Manuscript* manuscripts or any consequences arising from the use of any information contained in them.

RSC Publishing

www.rsc.org/loc

Registered Charity Number 207890

Recent Advancements in Optofluidics-Based Single-cell Analysis: Optical On-Chip Cellular Manipulation, Treatment, and Property Detection

Nien-Tsu Huang[1,2], Hua-li Zhang[3], Meng-Ting Chung[4], Jung Hwan Seo[5], and Katsuo Kurabayashi[6,7]

[1]Department of Electrical Engineering, National Taiwan University, Taipei, 10617, Taiwan

[2]Graduate Institute of Biomedical Electronics and Bioinformatics, National Taiwan University, Taipei, 10617, Taiwan

[3]School of Mechanical Engineering, Nantong University, Nantong, 226019, China

[4]Department of Biomedical Engineering, University of Southern California, Los Angeles, CA, 90007

[5]Department of Mechanical and System Design Engineering, Hongik University, 94 Wausan-ro, Mapo-gu, Seoul, 121-791, South Korea

[6]Department of Mechanical Engineering, University of Michigan, Ann Arbor, Michigan, 48109, USA

[7]Department of Electrical Engineering, University of Michigan, Ann Arbor, Michigan, 48109, USA

Lab on a Chip Accepted Manuscript

Abstract

Cellular analysis plays important roles in various biological applications, such as cell biology, drug development, and disease diagnosis. Conventional cellular analysis usually measures the average response from a whole cell group. However, the bulk measurement may cause misleading interpretations due to cell heterogeneity. Another problem is that current cellular analysis may not be able to differentiate various subsets of cell population, each exhibiting a different behavior than others. Single-cell analysis techniques are developed to analyze cellular properties, conditions, or functional responses in a large cell population at the individual cell level. Integrated optics with microfluidics platform provides a well-controlled microenvironment to precisely control single cell conditions and perform non-invasive high-throughput analysis. This paper reviews recent developments of optofluidics technology for various optics-based single-cell analyses, which involve single cell manipulation, treatment, and property detection. Finally, we provide our views on the future development of

integrated optics with microfluidics for single-cell analysis and discuss potential challenges and opportunities of this emerging research field in biological applications.

Introduction

Cellular analysis brings critical knowledge to modern biology and clinical sciences. An accurate understanding of cellular behaviors with in-depth information of their properties provides a scientific foundation for cell biology, drug development, and disease diagnosis. However, conventional cellular analysis only probes the average response from a whole cell group. It potentially poses two serious problems. First, cell-to-cell variability across the same type of cell group, or called cell heterogeneity, may yield misleading readouts from the cell group with diverse outputs.[1] Second, a specific cell type can have a few subsets. For example, T-cells are categorized into a few subsets, such as cytotoxic cells, helper cells, and regulatory cells, according to their distinct functions. Although each T-cell subset has a similar size, morphology, or biomarkers, it may express different proteins under various environments or stimulation conditions.[2, 3]

To address the above problems, single-cell analysis techniques have been developed to analyze various cellular functions in a large cell population at the individual cell level. A whole single cell-based assay typically entails three processes: (1) cell manipulation, such as cell trapping, selection or sorting,; (2) cell treatment, such as transfection, injection or lysis; and (3) detection of cellular physical and chemical properties, homeostatic conditions, or functional responses. As summarized in Table 1, single-cell analysis methods employ optical, mechanical, electrical, and other mechanisms, each exhibiting advantages and disadvantages. In particular, optical methods have become a major player in single-cell assay with recent advancements of commercial instruments and fluorescent biomarkers. For examples, optical tweezing and fluorescent activated cell sorting (FACS) are typically used for single cell trapping and sorting. Upon single cell treatment, DNA or nanoparticles can be precisely injected into a single cell using a pulsed laser. For single cell detection, microscopic imaging, flow cytometry, and enzyme-linked immunosorbent spot (ELISpot) are the most common techniques.

The optics-based methods find their wide use in single-cell analysis studies. However, they still require labor intensive and time-consuming procedures to localize an individual cell using complicated optical alignments, and bulky and sophisticated instruments. Besides, it is still challenging to control

Lab on a Chip Accepted Manuscript

environmental conditions while performing the optics-based single cell treatment or detection. The inability to control these conditions results in significant variability of the cell's fate and response. Then, how can we overcome these challenges? Synergistic integration of optical components with a microfluidic device, called "optofluidics," is a promising answer, providing unique advantages for the optics-based methods. Other non-optical techniques cannot necessarily share the same level of optofluidics-enabled advantages. This is the focus of our attention in this paper.

Optofluidics offers a unique platform enabling a rapid, high-throughput, non-contact single-cell analysis under a well-controlled spatial or temporal microenvironment. A microfluidic chamber can confine a single cell to an environment comparable to its size of 10-20μm in diameter.[4] A microfluidic channel network of appropriate fluidic design forms flow paths for reagents and single cells with a well-programmed timing and direction. These features enable precise control of cellular environments and processes in conjunction with optical manipulation and detection at the single-cell level. The notable advantage of fully optics-operated microfluidics for single-cell analysis is the simplicity of their platforms. Compared to other systems relying on electrical, magnetic or mechanical actuation/detection mechanisms, microfluidics enabled by free or embedded optics provides means to align, decouple, or exchange optical components according to cell sizes or cell analysis functions at greater flexibility. Moreover, the simple design of optofluidic systems allows for reducing the device fabrication cost and minimizing the device-to-device performance variation.

In this paper, we review optofluidic platforms for single-cell analysis, which have been demonstrated in recent studies. In general, these platforms are categorized based on the aforementioned three assay processes, namely, cell manipulation, treatment, and detection (Figure 1). With some of these platforms integrated together, we can envision future development of a comprehensive, multi-functional optofluidic system that covers the entire assay processes on a single chip (Figure 2). In this conceptual figure, three laser-light sources of different wavelength are aligned and share the same optical path to localize a target single cell at different positions of a microfluidic device. The target single cell is first trapped by a near-infrared laser and sorted to the cell treatment region. In this region, the cell gets optically transfected by DNA, RNA, or nanoparticles, or lysed using a pulse laser. Finally, the optically treated cell can be moved back into the main channel by the near-infrared laser and flow into the detection region. In the detection region, laser optics is used for fluorescent detection, optical imaging,

Lab on a Chip Accepted Manuscript

or label-free spectroscopy of the cell, depending on the interested cellular properties. We believe this kind of seamless process will reduce sample requirements, prevent potential contaminations, and reduce the total process time.

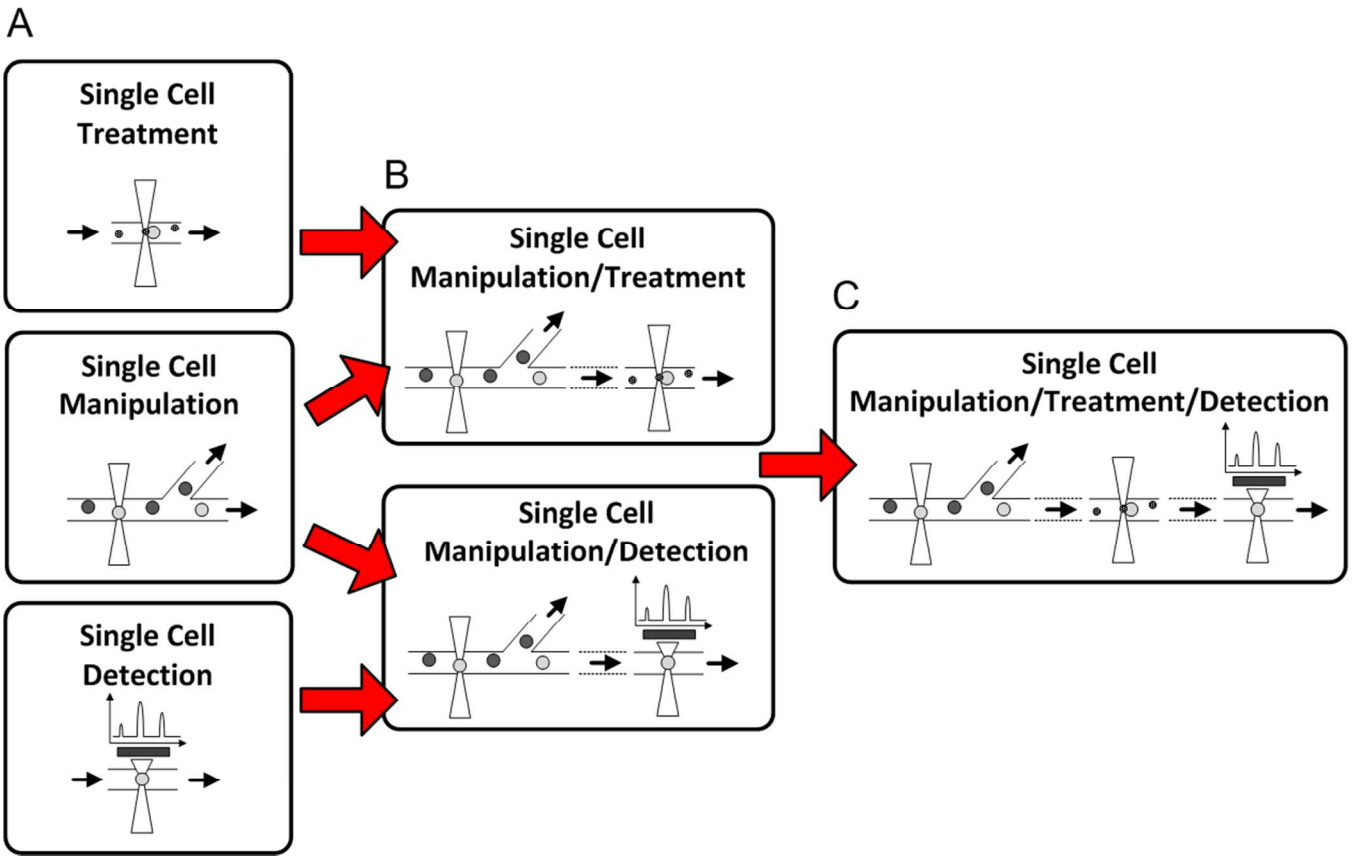

Figure 1 Optofluidic techniques for single-cell analysis. (a) Single cell manipulation, including cell trapping, selection, and sorting. (b) Microfluidic platforms integrate the single cell treatment or detection scheme after single cell manipulation. (c) Microfluidic platforms capable of systematically performing single cell manipulation, treatment, and detection.

Lab on a Chip Accepted Manuscript

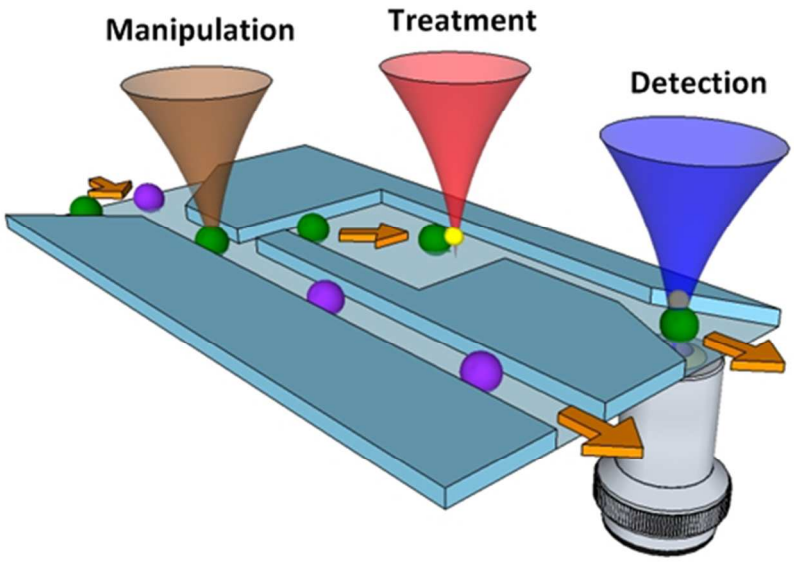

Figure 2 Conceptual illustration of an optofluidic platform performing a series of single-cell analysis processes, including manipulation, treatment, and property detection.

Lab on a Chip Accepted Manuscript

Table 1 The summary of single-cell analysis techniques enabled by optical, mechanical, electrical, and other forces and mechanisms.

	Single cell manipulation	Single cell treatment		Single cell detection
Optical force	Technique			
	Optical tweezers (gradient/scattering force)	Optical injection/transfection	Optical lysis	Optical stretcher, Raman tweezers
	Advantages			
	• High specificity and spatial resolution • Multi-parallel single cell array trapping	• High spatial resolution • Non-contact operation • Minimum cell damage by pulse light	• Fast • High specificity • Enable multiple functionalities (trapping and sensing)	• High Resolution • Long-term and dynamic cellular response monitoring is possible • Enable multiple functionalities (trapping and treatments)
	Disadvantages			
	• Cell damage due to high optical energy density • Low throughput (flow speed will affect trapping efficiency)	• Complicated optical setup	• Low throughput	• Cell damage due to high optical energy density • Low throughput
Electrical force	Technique			
	Dielectrophoretic force	Electroporation	Electrical Lysis	Electrical impedance measurement
	Advantages			
	• Ultra high throughput	• The ability to treat large cell population • Good transfection efficiency • Potential to integrated with other functions	• Fast	• Rapid response • Easy to analyze the data
	Disadvantages			
	• Local heating • Device complexity	• Large reagent required • Low cell viability	• Registrations on buffers • High electrical field is	• Low repeatability

Lab on a Chip Accepted Manuscript

			required	• Device complexity
Mechanical force	Technique			
	Hydrodynamic inertial flow	Patch-clamp	Mechanical Lysis	Atomic force microscopy (AFM)
	Advantages			
	• High throughput • Simple experimental setup	• High spatial resolution • Rapid response	• Flexible to various cell types • Simple chip design	• High sensitivity • High spatial resolution
	Disadvantages			
	• Difficult to control flow • Channel design is critical	• Hard to manipulate • Contact operation • Potential cell damage	• Low specificity • May cause partial lysis	• Low throughput • Microfluidics integration is not feasible
Other force (magnetic, chemical, thermal)	Technique			
	Magnetophoretic force		Chemical Lysis	
	Advantages			
	• High specificity • Multiple functionality • Less cellular microenvironmental impact		• Cheap and simple • High throughput	
	Disadvantages			
	• Weak force • Labeled magnetic nanoparticle is required		• May interfere with analytes	

Lab on a Chip Accepted Manuscript

1. Single Cell manipulation

Single cell manipulation in microfluidics usually requires exertion of an external force on the order of pN onto targeted cells. This type of force could be operated by electrical, magnetic, mechanical, or optical mechanisms (Table 1). Conventionally, the most widely adopted method is electrical force-driven single cell manipulation. This method utilizes dielectrophoretic (DEP) force applied to cells having dielectric material properties under a non-uniform electrical field. DEP force is strong enough to achieve accurate sorting of cells at high throughput. However, DEP operation may face sample heating and high power consumption, and requires extensive instrumentation. Mechanical force is another single cell manipulation method, which can directly position cells by shear stress in a liquid flow (e.g., sheath flow in flow cytometry). However, mechanical cell manipulation may require precise flow channel design and several flow control components, such as valves and pumps. To perform active cell sorting, electrical or mechanical methods usually accompany with fluorescence labeling to visually recognize target cells as is the case with fluorescence activated cell sorting (FACS). The need for both the labeling and manipulation of cells increases the complexity of sample preparation and instrumentation. Another form of labeling technique is achieved by binding magnetic nanoparticles with individual cells. These labeled cells are manipulated by means of force induced by a magnetic field. The advantage of magnetic field-activated cell sorting is the ability to simultaneously perform sensing and sorting for different cell types.[5] Setting a spatially well-defined magnetic field allows for constructing high-throughput cell sorter arrays with post-sorting analysis capability. Similar to magnetic sensing and sorting, another approach is to directly apply optics to both cellular detection and manipulation, leading to fully optical single cell sorting. The fully optical cell sorting technique is attractive since it is possible to realize multiple functionalities, such as cell trapping, manipulating, and sensing, with the same instrument. This makes the cell-sorting platform highly flexible and versatile.

Optical cell manipulation methods generally employ either gradient or scattering force. Gradient force, which acts as a pulling force on a dielectric particle, is proportional to the gradient of light intensity. With the ability to precisely control force for a large dynamic range, the gradient force-based manipulation technique is called “optical tweezers.” Scattering force, which acts as a weak repulsive force along the propagation direction, is proportional to the intensity of light. Yielding less damage to cells, the scattering force technique is suitable for stretching and rotating cells. In the following sections,

Lab on a Chip Accepted Manuscript

we briefly describe their theoretical backgrounds and review some optofluidic systems based on these two optical force-based cell manipulation mechanisms.

Gradient force

The optical tweezers-based single-cell manipulation starts with generating a large electromagnetic-field gradient at the focal point of a beam of light formed by a lens. This gives rise to an attractive force to pull a particle with its permittivity higher than the surrounding medium. Based on this pN-level gradient force, the focused light beam can act like tweezers to trap or move selected particles. Conventional methods incorporate steerable optical tweezers with an x-y stage to trap and move targeted particles. Compared to FACS, optical tweezers-based sorting has lower throughput due to the slow and limited movements of the stage and the mirror.

The first microfluidic flow cytometer capable of optically switching the flow direction of cells at high throughput was demonstrated by Wang *et al.*[6] (Figure 3a). The researchers first detected green fluorescence protein (GFP)-labeled cells labeled in the interrogation region of the cytometer. A high-intensity green-color fluorescent signal triggered an acousto-optic modulator (AOM) and activated a 20W CW laser to guide the GFP-positive cells into the collection channel by optical gradient force. The researcher verified a minimum stress perturbation and a recovery rate for the collected cells from their HSPA6/FOS gene expression after the sorting process. More recently, Werner *et al.*[7] demonstrated the use of an array of optical tweezers to trap up to 200 individual yeast cells in parallel within a microfluidic cytometer channel (Figure 3b). After trapping individual cells in an array form, the researchers transported the trapped cells to another side of the microfluidic channel filled with reagents and then monitored their response. Finally, the cells of interest were optically guided into the sorting channel by the same steerable tweezers. Due to minimum cellular damage achieved by the appropriate optical density applied to cells, this optical tweezers-based single cell trapping array optofluidic system allowed the researchers to study the long-term and dynamic cellular response to a change in the environmental condition and functional heterogeneity across the trapped cells to obtain a statistically significant result.

Scattering force

Lab on a Chip Accepted Manuscript

Another common strategy is the use of scattering force induced by a laser beam to manipulate cells to a wanted area or channels. For example, Kim *et al.*[8] demonstrated a technique so called the "cross-type optical particle separation (COPS)." The COPS technique employs a slightly focused laser beam to push single cells or particles. As shown in Figure 3(c), the researchers first flew cells in a microfluidic channel which is orthogonal to the direction of laser beam. When passing the laser beam, the cells were pushed to a direction parallel to the laser beam by scattering force and displaced from their original trajectories. Compared to traditional gradient force-based optical tweezers, the COPS technique required much less laser power and energy density at the focal point, which could minimize potential cellular damage by the laser beam. For example, Bragheri *et al.*[9] demonstrated a COPS-type optofluidics cell separation using a femtosecond laser. The integrated design made the device compact and potentially suited for multifunctional operations with less cellular damage. Lee *et al.*[10] further tested the mobility of four different blood cell types with the COPS setting and demonstrated potential use of the technique for label-free separation of blood cell subpopulations (Figure 3d). Another type of optical scatting force-based single-cell sorting was demonstrated by Kovac *et al.*[11] The researchers proposed an optical image-based microfluidic cell sorter incorporating microwell arrays, each trapping a single cell (Figure 3e). With this device, they inspected the individual cells using fluorescence signals taken from a whole-cell or subcellular image across the entire arrays. After the inspection, the researchers applied optical scattering fore to the trapped cells to push them out of the microwells and flew them out. This image-based cell sorter enabled sorting of cells according to the both spatial and temporal information of subcellular conditions.

Although optical scattering force is not as strong as gradient force to manipulate cells, the technique is easier to operate and readily integrated with a microfluidics setting. Thus, it can potentially permit cell manipulation with a higher degree of freedom, and one can find a wider variety of optofluidic designs using optical scattering force.

Optically induced mechanical and electrical force

Instead of directly using optical force, researchers also demonstrated using optically induced mechanical and electrical force to perform single cell manipulation. For example, Chen *et al.*[12] proposed a device called the "3D pulsed laser triggered fluorescence activated cell sorter (PLACS)" (Figure 3f). They used a high power pulse laser to generate a micro bubble near the junction of the device. The bubble induced

Lab on a Chip Accepted Manuscript

a perturbing flow that pushed a selected cell to the collection channel. The PLACS device achieved cell sorting at a throughput as high as 23,000 cells/second. In addition, the pulse-laser induced mechanical force could achieve a ultra-fast single cell switching rate permitting on-off cycles of 20 μs period. Chiou *et al.*[13] first proposed an optofluidic device in which single cells were manipulated by light-induced dielectrophoretic (DEP) force, namely "optoelectronic tweezers (OET)." Their device incorporated a photoconductive multi-layer structure consisting of an optically transparent indium tin oxide (ITO) layer and a hydrogenated amorphous silicon layer. This multi-layer structure acted as a virtual electrode driven by a pattern of projected light and induced non-uniform electrical fields in the microfluidic chamber of the device. Adjusting the two-dimensional electrodes pattern, the researchers demonstrated massively parallel manipulation of single cells by light-induced DEP force. However, a drawback of the conventional OET system is its complex device architecture, which makes it challenging to integrate the system with a microfluidic unit for subsequent single-cell analysis. To address this issue, Huang *et al.*[14] used single-wall carbon (SWNT) nanotube electrodes in their OET optofluidic device. The use of SWNT electrodes integrated in a microfluidic channel made the device fabrication simpler and the device design more flexible (Figure 3g). The device consisted of an ITO/amorphous-silicon photoconductive layer deposited on the bottom surface and SWNT electrodes embedded on the top surface of the channel. The researchers first identified a desired cell from its fluorescence signal, trapped it by DEP force generated by a light spot, and then moved it with the light spot to an isolated chamber. Multiple cells could be manipulated in parallel with light spots at a speed of 10 μm/s. Finally, the isolated cells were collected separately for downstream analysis. By applying the SWNT electrodes, the OET technique could extend its flexibility to implement various microfluidic systems for other single cell assay processes than cell manipulation.

Lab on a Chip Accepted Manuscript

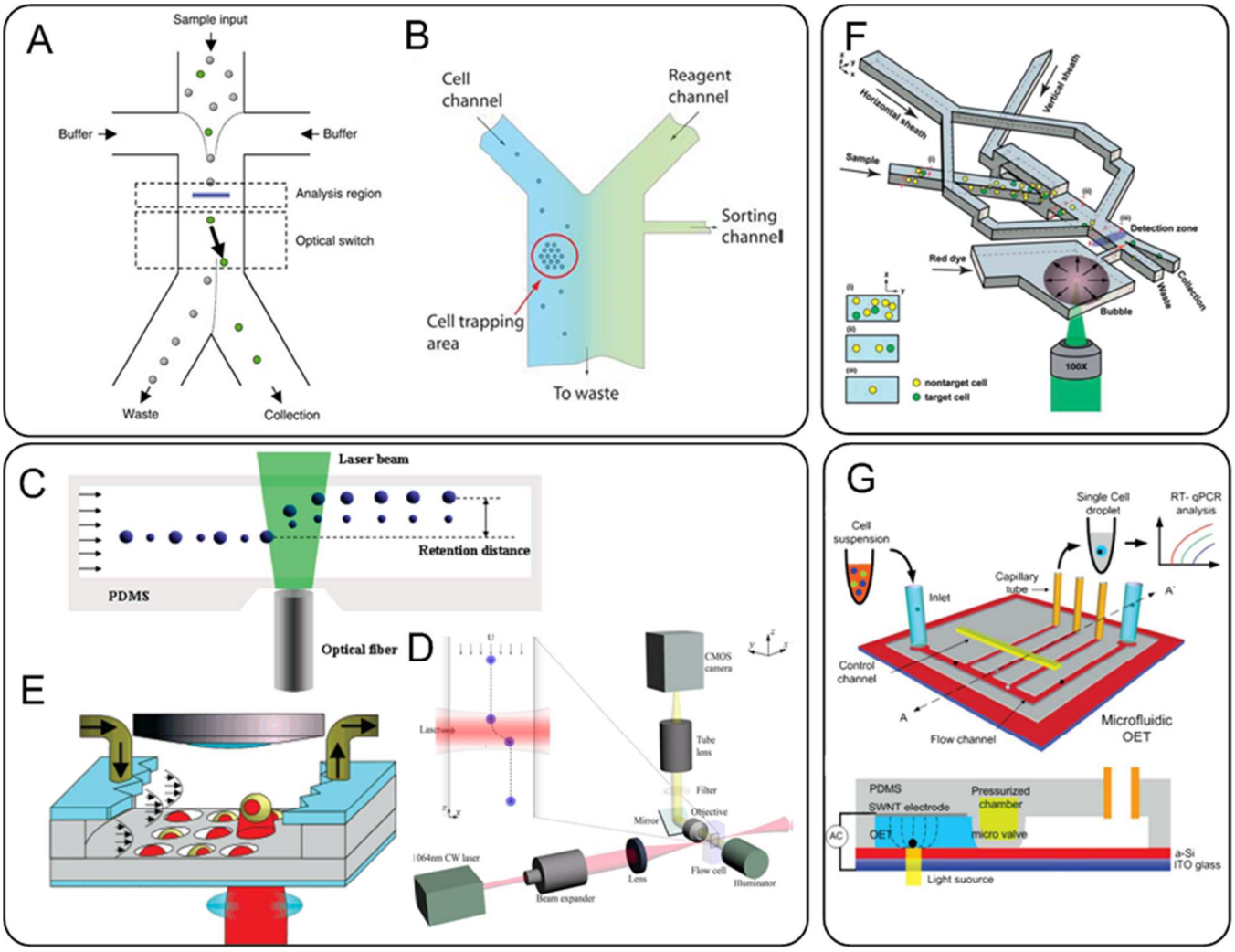

Figure 3 Optofluidic single cell manipulation by optical gradient force: (a) single cell sorting by optical tweezers[6] and (b) optical tweezers trapping array[7]. Optofluidic single cell manipulation by optical scattering force: (c) optical scattering sorting (embedding fiber),[8] (d) optical scattering sorting (free space optics),[10] and (e) optical scattering array.[11] Optofluidic single cell manipulation by optically induced force: (f) optical induced bubble sorting (mechanical force)[12] and (g) optical induced electrical field sorting (electrical force).[14] Reproduction of the figures has been made with permissions from Nature Publishing Group, Royal Society of Chemistry, American Chemical Society and American Institute of Physics.

Lab on a Chip Accepted Manuscript

2. Single cell treatment

Cell treatment comes into play as a process subsequent to trapping and sorting of targeted cells. Similar to cell manipulation, cell treatment can also be performed by various force mechanisms. Table 1 summarizes advantages and disadvantages of single cell treatment enabled by various methods. In this section, we specifically focus on optical transfection, injection, and lysis as cell treatment processes. Transfection and injection are typically defined as processes of introducing membrane-impermeable biomolecules and materials into a cell and examining their subsequent expression of a desired intracellular protein. The terminology usage between "transfection" and "injection" depends on the type of materials optically introduced to a cell. If the introduced material is DNA, RNA, protein or nucleic acid, it is called optical transfection. With impermeable compounds, such as organic fluorophores or nano-particles, inserted into cells, it is called optical injection. There are numerous applications of cell transfection and injection in agricultural biotechnology,[15] cell biology,[16] gene therapy,[17] and drug development.[18, 19] For example, GFP can be transfected into cells to identify the spatial location of interested gene expression. The diabetes disease can also be treated by introducing a modified gene into cells.[20] Optical tweezers-based injection method uniquely offers higher spatial and temporal accuracy in placing a nanoparticle into a single cell than biological processes, such as phagocytosis and endocytosis, and physical processes based on mechanical and electrical mechanisms. In addition to transfection and injection, optical lysis is another cell treatment process which applies optical energy to a cellular membrane and breaks down a cell to collect its intracellular contents. Optofluidics provides a well-controlled microenvironment to trap a single cell and a precise optical spot to perform optical cell treatment. In the following section, we provide several examples of optical transfection, injection and lysis achieved in an optofluidic setting.

Optical Transfection and Injection

Researchers have used transfection methods to study cellular response under modified gene expression or enhance localized molecular imaging. Previous transfection techniques include carrier-based methods (e.g., calcium phosphate transfection,[21, 22] lipid-mediated transfection,[23-27] viral vectors,[28-31] nanoparticle-assisted transfection,[32-34] and etc.) and membrane permeabilization methods (e.g., electroporation,[35-38] sonoporation,[39-41] and etc.) as well as direct transfer methods (e.g., gene guns,[42-44

Lab on a Chip Accepted Manuscript

and microinjection,[45-47]). However, none of them achieves high efficiency in targeted DNA transfer in a non-contact, non-toxic, and stable manner. These methods are unable to achieve *in situ* evaluation of transgene expression. Moreover, most of these techniques yield adverse effects, such as unexpected morphologies and abnormalities in transfected cells.

Over the last few decades, several laboratories have developed laser-mediated gene transfection techniques, known as optical transfection. Compared to other transfection methods, optical transfection is the most promising non-disruptive technology for gene therapy and DNA manipulation in targeted specific cell(s) at high efficiency and reliability. Optical transfection is a process using laser light focused on a cell membrane to generate a transient hole through which membrane-impermeable substances are allowed to pass into the cell (Figure 4(a)). The laser light focused into a submicron hole on the cell membrane using a high numerical aperture (N.A.) objective lens creates minimum cell damage compared to other methods. A high-repetition rate (80 MHz) femtosecond laser operating in the near-infrared region (~ 800 nm) is the most reliable and promising light source to permeabilize the cell membrane. A long train of low-energy femtosecond laser pulses results in higher post-irradiation cell viability and more stable transfection than other laser sources,[48, 49] such as UV pulsed laser,[50] laser in the visible wavelength band,[51] and single femtosecond pulse laser.[48, 52, 53] Owing to its capability to enable single cell selectivity, localized delivery, non-toxicity, and consistency, femtosecond laser-based optical transfection incorporating two-photon scanned microscopy are widely used in biological research laboratories for *in vivo* applications.

Another recent trend is to combine optical tweezers (CW laser) and optical injection (Pulsed laser) using the same light source with its operation mode adjusted by a minor configuration change. Brown *et al.*[54] demonstrated both optical trapping and injection using a 800nm Ti: Sapphire laser by switching CW and pulsed modes for a single cell. They first used a CW mode to tweeze a target cell into a Trypan Blue-filled capillary tube. Then, they changed the laser into the pulse mode to optically create a transient pore for staining the cell nucleus with the Trypan Blue dye. They finally switched the laser back to the CW mode and moved the cell to its original position. Thus, this study simultaneously achieved optics-based single cell trapping and injection. Besides fluorescent molecules (e.g. GFP, Propidium iodide (PI), and Trypan Blue), researchers demonstrated optical injection of a metal nanoparticle into a target live cell. The injection of gold nanoparticle into a single cell can be used for bio-imaging, single molecule

Lab on a Chip Accepted Manuscript

tracking, biosensing, drug delivery, transfection, and diagnosis. One can refer to a critical review by Levy *et al.*[55] for more detailed information on research in this field. One of the most representative studies was conducted by Mcdougall *et al.*[56] They developed a customized microscopy system that allowed for trapping and injecting a 100nm-gold nanoparticle. Figure 4b shows the three-dimensional position of a gold nanoparticle (red color) inside a single Chinese hamster ovary cell (green color). They confirmed the internalization of the nanoparticle within the cell by confocal microscopy imaging. This technique can potentially be used for single-cell surface enhanced Raman scattering (SERS) measurements.

Despite the promise shown by previous studies, low-throughput, inconsistent, and labor-intensive single cell trapping operations limit the conventional optical transfection and injection approaches.. Overcoming these shortcomings still poses a significant challenge and remains less explored. With recent advancements in microfluidic technology, some researchers have made initial steps toward addressing this challenge. For example, Marchington *et al.*[57] developed an integrated optofluidic platform for automated single-cell optical injection operation with high sample-loading throughput (Figure 4(c)). This platform enabled the researchers to optically inject PI into HEK 293 mammalian cells with an injection efficiency (or injection success rate) of 42±8% and a sample-loading throughput of 1 cell/second. They subsequently obtained fluorescence images of the treated cells with Calcein AM and observed a cell viability of 28±4% with these cells. Another study by Rendall *et al.*[58] introduced a two-dimensional flow focusing mechanism and a diffraction-free Bessel beam to further improve the single-cell nanoparticle injection efficiency. The researchers also achieved an increased throughput by generating "photopore" arrays with a multi-parallel flow/laser channel architecture.

Lab on a Chip Accepted Manuscript

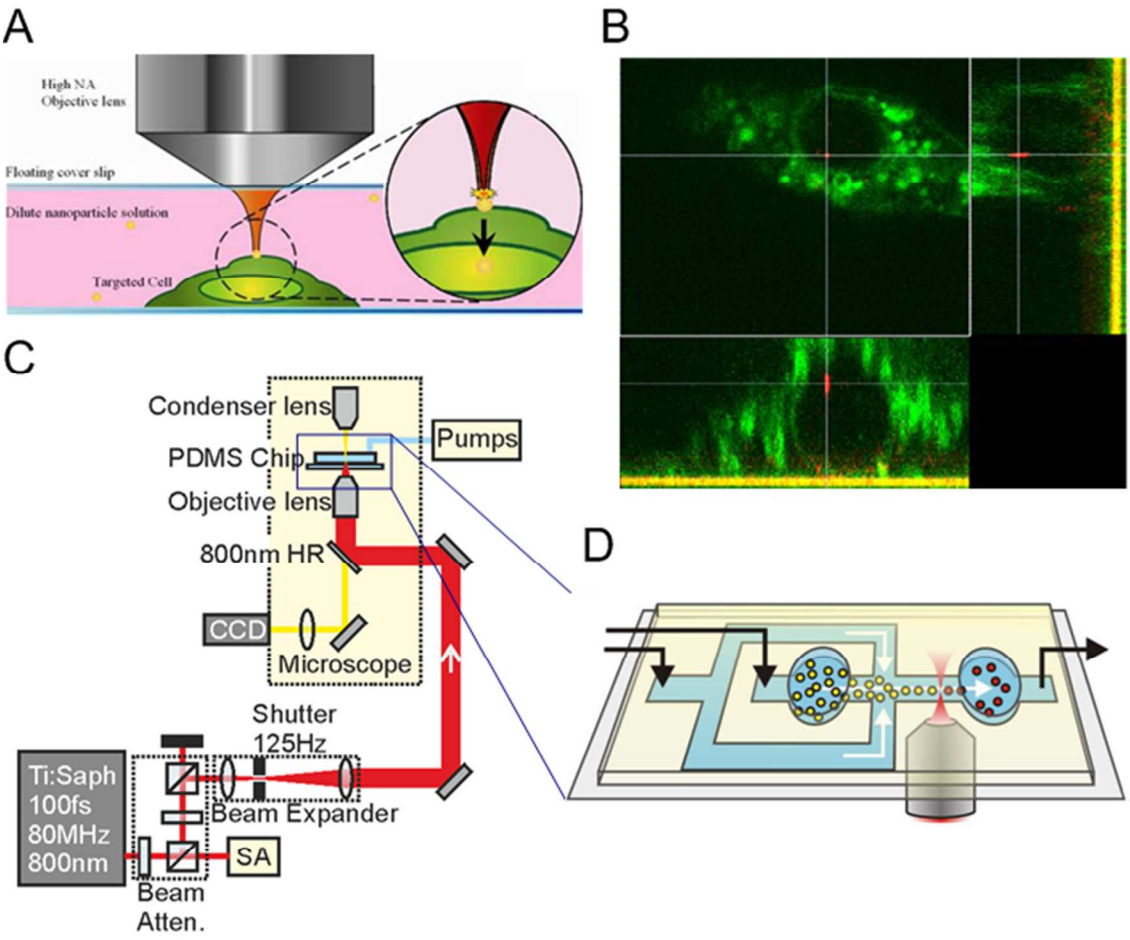

Figure 4 (a) Single nanoparticle injection using a high numerical aperture (N.A.) microscope objective. (b) 3D fluorescent image showing the location of a gold nanoparticle (red color) inside a single Chinese hamster ovary cell (green color).[56] (c) Optofluidic platform for optical injection. (d) Cell loading to PDMS microfluidic channel and the femtosecond laser beam focusing for optical injection.[57] Reproduction of the figures has been made with permissions from John Wiley and Sons and The Optical Society.

Optical Lysis

Cell lysis is another cell treatment process, which breaks down the impermeable cell membrane and releases intracellular macromolecules and organelles for further examination. Two conditions need to be met to perform efficient single cell lysis. First, the process has to be performed in a well-controlled microenvironment to efficiently isolate a cell with its lysate. In this case, microfluidics provides an ideal

Lab on a Chip Accepted Manuscript

platform to perform single cell lysis operation in a confined space. Accordingly, quite a few studies have employed a microfluidic system to achieve cell lysis based on either a mechanical,[59, 60] thermal,[61] chemical,[62, 63] electrical[64] or optical[65] process. Table 1 summarizes advantages and limitations of each single cell lysis technique. Second, the process has to be quick enough to prevent any interference to cell signaling, which usually happens within a second and is also sensitive to environmental conditions change surrounding cells.

Generally, chemical lysis using detergents is the most common approach to break up the cell membrane. However, it is usually a quite slow process and may affect the cell signaling functions during lysis. Electrical lysis is one of alternative methods to avoid this problem, owing to its much faster lysis speed. Yet, this method requires a large electrical field at a high frequency. Besides, the buffer selection is also limited in the electrical lysis process. Moreover, the electrical lysis setup inherently requires microelectrode integration in a microfluidic system by sophisticated fabrication, thus leading to high device manufacturing costs. In contrast, laser pulse-induced cell lysis technique is more advantageous as it rapidly breaks up the cell membrane with less reagents and device configuration constraints. The pulsed laser yields high temporal and spatial resolution for single cell lysis. Furthermore, it can easily be focused or steered to any position on the targeted cell placed in an optically transparent microfluidic channel. In addition, adjusting the focal position or intensity of the laser enables the optical lysis process to be readily converted into other processes, such as optical mixing, transfection, and injection.

The optical lysis process starts with inducing plasma (i.e., ionized vapor) in solution by a focused laser beam (Figure 5a). The plasma subsequently results in the generation of either shock waves or cavitation bubbles which cause cell rupture.[54] Quinto-su *et al.*[65] studied the plasma formation and cavitation bubble dynamics of optical lysis of non-adherent BAF-3 cells by capturing high-speed fluorescent images with a gated intensified CCD camera (Figure 5b). They observed the completion of the lysing process 95-239ns after the laser irradiation and confirm this rapid process could minimize the dispersion of intracellular contents. Using an optofluidic setting, Phillips *et al.*[66] demonstrated they can sequentially perform on-chip optical lysis and single mammalian cells analysis with the electrophoresis method (Figure 5c). The researchers first loaded the cells with two intracellular fluorescent species: fluorescein (FL) and Fluorescein carboxylate (FL(COOH)). Then, they optically lysed cells by the picosecond-pulsed laser after flowing them into the microfluidic channel. Finally, the fluorescent lysates

Lab on a Chip Accepted Manuscript

(FL and FL(COOH)) inside the cell were separated by electrophoresis and detected by the photomultiplier (PMT) connected with optical fibers.

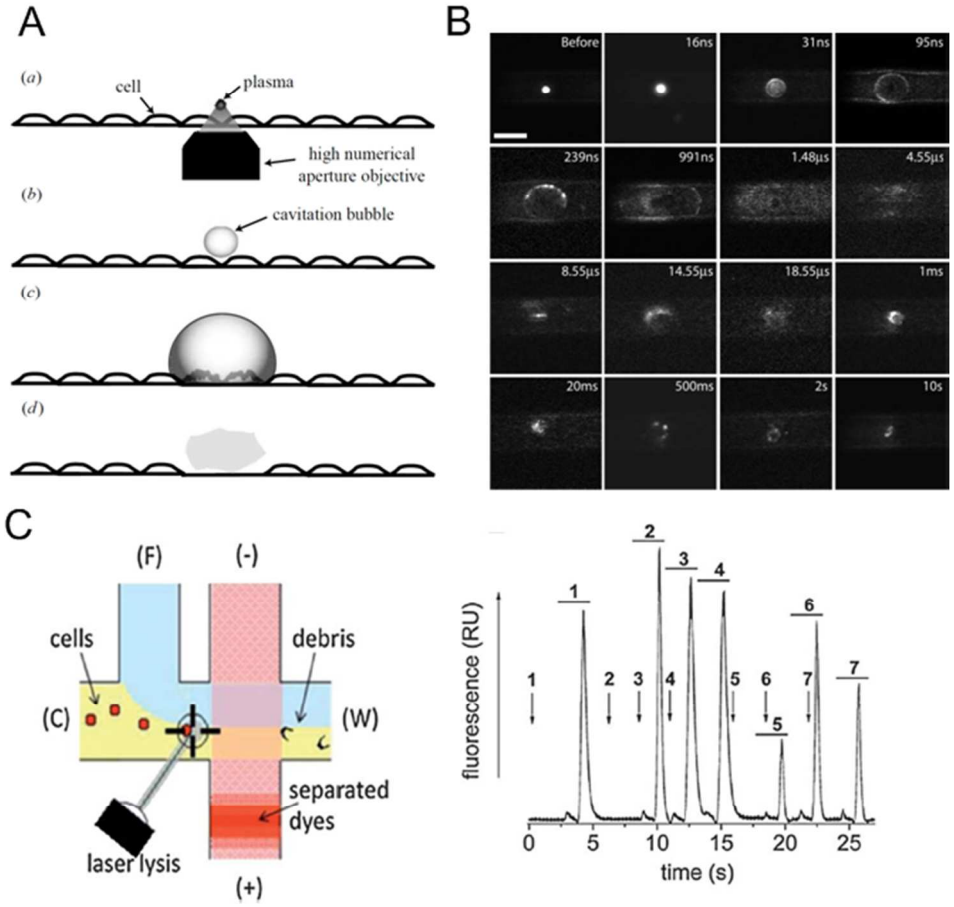

Figure 5 (a) Steps of laser-induced cell lysis process.[54] (b) Time-lapsed fluorescent images showing cell lysis dynamics in microfluidic channel.[65] (c) (left) Optofluidic device enabling cell lysis and electrophoresis separation of two fluorescent dyes loaded inside the cell on the same chip platform. (right) Electropherogram from 7 individual cells. The larger peaks with an underlined number represent signals from the FL dye while the smaller peaks with a number having no underline were obtained from the FL(COOH) dye.[66] Reproduction of the figures has been made with permissions from Royal Society Publishing and Royal Society of Chemistry.

Our review reveals that optofluidics-based single cell lysis is the most suitable approach to meet the aforementioned two conditions: (1) suitable microenvironment and (2) high-speed operation. In addition, the optical lysis process could be easily integrated with other single-cell analysis processes in the same optofluidic platform with a simplified design. Enabling continuous procedures, this would potentially

Lab on a Chip Accepted Manuscript

improve the throughput of single-cell analysis.[67] Achieving high throughput and accuracy in single cell lysis currently still poses major challenges.[68] But we believe that integrated optofluidics permitting the simultaneous implementation of cell lysis and quantitative detection of single-cell level organelles and biomolecules can open up new possibilities to overcome these challenges.

Lab on a Chip Accepted Manuscript

3. Single Cell Detection

Biophysical and biochemical properties of living cells are both important parameters to determine cells' physiological conditions. The relationship between human diseases and cell physiological conditions has been studied in recent decades. Previous researchers found that abnormal biophysical and biochemical properties of cells may alter the cytoskeleton composition,[69] reorganize the network structure,[70] change the protein density,[71] and may eventually lead to cell death or severe diseases. Biophysical properties, including cell viability, size, shape, stiffness, deformability, and refractive index are important parameters to identify individual cell conditions. Biochemical properties, such as protein secretion, calcium levels, and surface biomarkers are used to understand cellular functions or responses to environmental conditions.

Characterization of the biophysical and biochemical properties of single cells usually relies on mechanical, electrical, and optical detection methods (Table 1). For example, atomic force microscopy (AFM) is a mechanical characterization technique that allows for studying the mechanical properties of single cells. AFM measurements use a micrometer-scale cantilever beam and tip to contact the cell and measure its stiffness.[72, 73] However, the throughput of AFM is usually very low with their scanning time easily exceeding several minutes for each cell, which make it unsuitable for microfluidic integration. Another method of studying single-cell biophysical properties is based on electrical impedance measurements, which allows researchers to characterize the ingredients of a single cell.[74] However, this method needs to integrate electrodes in a microfluidic channel, which increases the cost and complexity of the device. In contrast, optical methods are much easier to implement in a microfluidic system. Optical methods typically used for single-cell analysis include optical imaging,[13, 75] interferometry,[76] and optical spectroscopy,[77-79] which are categorized by whether they involve fluorescent labeling or label-free biodetection.

Optofluidics provides an ideal platform to study the biophysical and biochemical properties of single cells as it permits rapid, simple, sample/cost-effective and non-invasive measurements. Recently, we have seen significant advances in optofluidic technology for cellular biophysical and biochemical property detection by a wide variety of optical methods. One can find our review on this topic somewhere else.[80] In this paper, we are particularly interested in optofluidics technology enabling integration of optical biodetection with other optics-based cell trapping, sorting, or treatment in a

Lab on a Chip Accepted Manuscript

common microfluidic platform. Thus, we focus our review on studies specifically related to multi-functional optofluidic integration for single-cell property analysis. We discuss optofluidic cell deformability assay and Raman Tweezers as the most illustrative examples.

Cell deformability detection

The elasticity of cell is an important biomarker which offers a sensitive alternative to current proteomic techniques for basic cell biological investigation, cell classification, and disease diagnosis. Thus, measurement of cell deformability in microfluidics has been used for diagnosis of cell-related diseases. For example, cell deformability is found to be sensitive during the progression of cells from a normal to cancerous and even metastatic state (Figure 6(a)).[81] Cancer cell lines are more deformable than normal cells. The viscoelastic deformability of malignantly transformed SV-T2 fibroblasts has been measured significantly higher than normal BALB/3T3 fibroblasts.[69] Microfluidic devices for cell deformability measurements are based on how the cells are deformed under channel-based constriction, fluid stress, optical stretching, electro-deformation, electroporation, and aspiration.[82-84] The optical cell manipulation techniques discussed in single cell manipulation part (optical gradient or scattering force) enable these cell deformability measurements.

Among these methods, optofluidics technology combining optical tweezers and label-free optical imaging with a microfluidic structure has become a promising tool. The multi-functional optofluidic platform allows researchers to investigate cell behavior and response under physical and chemical stimulation. A typical optical stretcher system consists of a microchannel and two laser fibers located on the sides of a passageway without contacting the cell under study.[85] If the cells are not centered on the optical axis of beam, a restoring force is exerted to keep cells on the optical axis. Microfluidic systems incorporating the integrated dual-beam optical stretcher are very promising for high throughput optical manipulation of individual cells, coupled with several other functionalities, such as accurate cellular sorting, trapping, and multiple parallel analysis of cell mechanics.

Lab on a Chip Accepted Manuscript

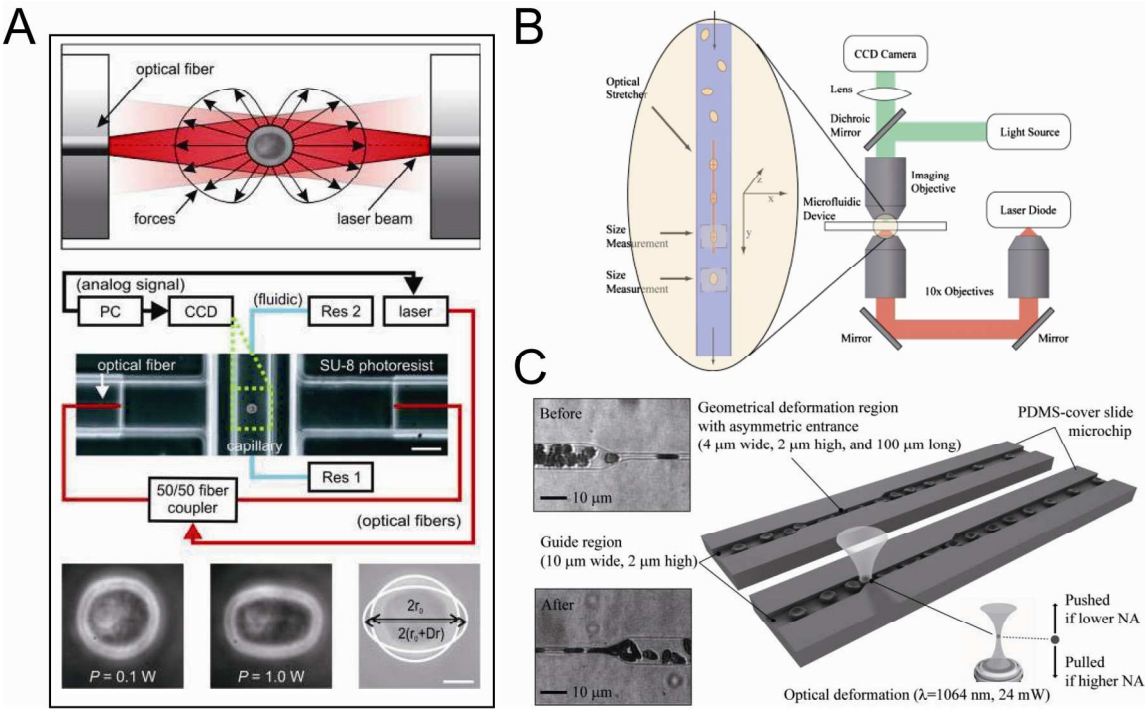

Figure 6 Schematic of cell deformability technique using optical stretcher. (a) Optical stretcher with two-counter propagating laser beams emanating from optical fibers (embedding fiber).[81] (b) Optical deformation experiment setup using laser diode (free space optics).[86] (c) RBCs deformability was measured with construction channel under optical pressure for enhancing detection sensitivity.[87] Reproduction of the figures has been made with permissions from National Academy of Sciences, USA, John Wiley and Sons and Royal Society of Chemistry.

Researchers also used a simple asymmetric optical trap by a single-beam diode laser to optically stretch RBCs in a microfluidic environment,[86] as shown in Figure 6(b). They conducted both optical deformability experiments and numerical simulations to quantify cell elastic parameters and investigated cell relaxation dynamics in repeated stretches. One of the other interesting applications of cell deformability measurement is to analyze erythrocyte deformability for detection of hematological disorders, since erythrocytes are highly deformed in a microenvironment under optical force. So the biophysical properties of those cells are used to be a critical marker of disease detection factors with loss of deformability and a change of blood viscosity.[88]

Lab on a Chip Accepted Manuscript

To conduct on-chip cell deformability tests, Lee *et al.*[87] developed an asymmetric PDMS channel coupled with an optical tweezers type. In order to improve the test performance, optical pressure was employed with the asymmetric entrance of the deformation region (Figure 6(c)). The study demonstrated that a 3D distribution of geometry-sensitive parameters (e.g., transit velocity and elongation index) and optic-sensitive parameters (e.g., shape recovery time) provided clear information on the difference of erythrocyte deformability between normal and cancerous red blood cells. Another example of using a microfluidic optical stretcher is found in oral cancer diagnosis. Remmerbach *et al.*[89] observed a difference between the mechanical behavior of oral squamous cancer cells and that of the primary cells extracted from healthy donors.

Raman tweezers

Raman spectroscopy is a label-free, non-destructive optical detection technique that allows for obtaining the spectral signature of the chemical composition of a cell organism. Raman spectra provide information on the vibrational modes of intracellular molecules resulting from light-molecule interactions. A technique called "Raman tweezers," which combines optical cell manipulation with Raman spectroscopy, has successfully been used for discriminating biotic and abiotic particles,[90, 91] unveiling protein expression information on living bacteria,[92] sorting cells after Raman-based identification,[93] and detecting hyperosmotic stress in trapped single yeast cells.[94] Raman tweezers enable discrimination between normal blood cells and circulating tumor cells[95, 96] with higher specificity than the optical stretcher methods described above. The spectral intensity of specific wavelength represented particular intracellular proteins and nucleic acids of cancerous cells, such as colorectal epithelial cells, prostate cancer cells, cancerous hematopoietic cells, and astrocytoma, is known to be higher than that of normal cells.[97-100]

Raman tweezers operations can be highly facilitated in an optofluidic platform, where optical trapping waveguides can be precisely pre-aligned with a microfluidic channel with reagent flows precisely controlled. For example, Snook *et al.*[101] employed an integrated optofluidic setup for Raman Tweezers (Figure 7(a)), by which researchers performed separation and trapping of single cells and then optical sorting of the cells according to their Raman spectral signatures. The researchers demonstrated Raman signal-activated sorting of leukocytes and other three tumor cell lines (OCI-aML3, MFC-7 and BT-20)

Lab on a Chip Accepted Manuscript

based on their Raman spectra (Figure 7(b)). Their optofluidic platform permitted incorporation of different optical paths for trapping and Raman excitation. This device configuration enabled the detection of resonance Raman spectra of a trapped single cell at different excitation wavelengths.

Raman tweezers combined with microfluidics can capture cellular responses caused by drug delivery or environmental stimulation in real time. Ramser *et al.*[102] observed the oxygenation dynamics of single red blood cell (RBC) using Raman tweezers. The researchers suspended the cell in solution while optically trapping it in a microfluidic channel with a well-controlled stimulant reagent flow. They detected the time-variations of Raman spectral peaks, which resulted from the selective vibrational resonance of the porphyrin groups of the RBC hemoglobin, as shown in Figure 7(c). This optofluidic platform allowed the researchers to perform *in-vivo,* dynamic characterization of the oxygenation cycle of the single RBC for a long term.

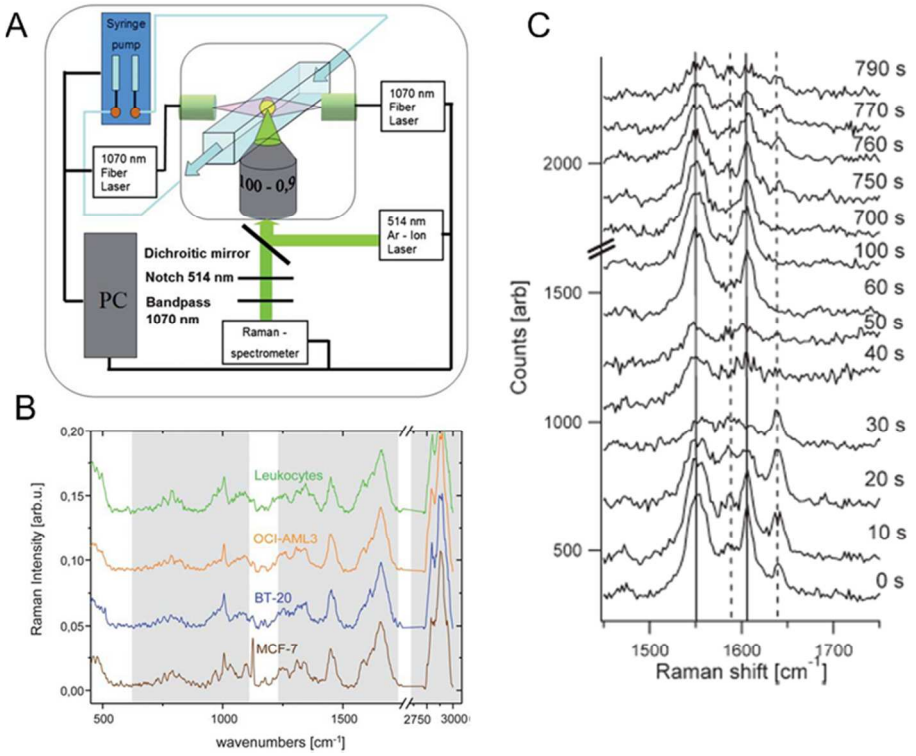

Figure 7 Raman tweezers. (a) optical setup (b) comparison of Raman spectra between leukocytes and other three tumor cell line (OCI-aML3, MFC-7 and BT-20).[101] (c) Raman tweezers for oxygenation cycle of single red blood cell.[102] Reproduction of the figures has been made with permissions from Royal Society of Chemistry.

Lab on a Chip Accepted Manuscript

One of the current challenges in designing a Raman tweezers system is to minimize potential harm to cells by high-power, high-intensity excitation laser light. This harm may result from photochemical and photothermal damages, bubble formation, photomechanical stress, and acoustic wave formation. In particular, photothermal and photochemical damages have been recognized as dependent on different laser wavelengths.[101] Much care is needed for selecting the Raman excitation wavelength to prevent the damages. The optofluidic platform incorporating the aforementioned dual optical-path configuration provides flexibility in Raman wavelength selection by allowing it to be separated from the trapping wavelength. Further advances could be made to integrate a nanoparticle injection function in the same optofluidic system. With gold nanoparticles optically injected into cells, the Raman signals could be significantly enhanced by means of Surface Enhanced Raman Scattering (SERS). This would eliminate a need for high-power, high-intensity excitation light, thus preventing the photodamages.

Lab on a Chip Accepted Manuscript

Table 2 Recent developments in optofluidics-based single-cell analysis

Functions	Methods	Mechanisms	Discriminating Parameters	Power / Time /Wavelength	Throughput	Targeted cell/Biological problem	Ref
Cell Manipulation (Sorting)	Optical gradient force	The target cells can be moved and sorted by gradient force of optical tweezers.	Fluorescent markers	20W/<10ms/ 1070nm	100cells/sec	HeLa,RAW264.7, yeast cells, mitochondrion, bacillus, protoplast	6
	Optical scattering force	The target cells can be moved by scattering force of focused laser beam.	Optical mobility	3W/-/1064nm	-	RBCs, Lymphocytes, Granulocytes, Monocytes	10
	Optical scattering force	Using integrated fiber optics design.	Fluorescent markers	5W(350mW in channel)/1sec/1040nm	Up to 50cells/minute	Human cells	9
	Optical scattering force	Using optical scattering force to remove cells from array and flow out.	Fluorescent markers	125mw/~10s/980nm	100-300cells/hour	epithelia breast cancer cel(MCF7)	11
	Others (Pulsed laser induced bubble)	The target cell will be moved by perturbation flow generate by pulsed laser induced bubble.	Fluorescent markers	31uJ/8ns/532nm	Up to 20000 cells/sec with 37% purity, 1500~3000 cells/sec with > 90% purity.	mammalian cells(Nalm-6, Ramos)	103
	Others (OET)	Induce non-uniform electrical field to guide cell	Fluorescent markers	10V/-/633nm@300Hz	Flow speed: 10μm/s	HeLa Cells Ramos cells	14
Cell Manipulation (Trapping)	Optical gradient force	The target cells can be parallel trapped and sorted by optical tweezers.	None	10W(30mw each)/-/1064nm	2-3 cells/minute	Yeast cells	7
Cell Treatment	Optical injection	Multi-photon effect; generation of low density free electron plasma cloud	None	2W/100fs/800nm@80 MHz	1cells/sec	HEK 293 mammalian cells	57

Lab on a Chip Accepted Manuscript

	Optical injection	Multi-photon effect; generation of low density free electron plasma cloud	None	1.8W/140fs/800nm@80MHz	10cells/sec	HL-60 CHO-K1	58
	Optical lysis	Laser-induced plasma followed by shock wave emission and cavitation bubble formation	None	4.63μJ/540ps/532nm	-	BAF-3 cell	65
	Optical lysis	Laser-induced microcavitation,	None	14.1μJ/6ns/1064nm	1μL/min, 1-4cells/load	BE colon carcinoma cell line, MDA-MB-468 breast cancer cell line	104
Cell Manipulation integrated Detection	Cell deformability	Optical scattering force	Cell Stiffness	0.1W(trap)1W(deform) /-/1064nm	-	Acute promyelocytic leukemia (APL) cells	81
	Cell deformability	Optical gradient force	Cell Stiffness	200mW/-/830nm (stationary cells) 5W/-/808nm (cells in flow)	100cells/sec	Red blood cells	86
	Cell deformability	Optical gradient force	Cell Stiffness	24mW/-/1064nm	-	Red blood cells	87
	Raman Tweezers	Optical gradient force	Oxygenation cycle	90-120mW/-/1064nm	200-600s for long term monitoring	Red blood cells	102
	Raman Tweezers	Optical scattering force	Cell membrane profile	5W/-/1070nm	5 cell types/10s	OCI-AML3, MFC-7 and BT-20, (leucocytes and erythrocytes are control)	101

Lab on a Chip Accepted Manuscript

4. Outlook

In this paper, we have critically reviewed recent developments in optofluidics-based single-cell analysis (Table 2). Our review indicates that optofluidics technology holds promise to facilitate integration of multiple single-cell assay processes into a single chip. All the assay processes on the chip could be operated by optics, and we call such a system a "fully optical microfluidic (FOM)" platform. A good example is the platform developed by Salehi-Reyhani *et al.*[104] (Figure 8). They used three laser light sources to enable single-cell trapping, lysis, and protein detection all together on the common optofluidic chip (Figure 8b). One of the light sources was a 1070nm CW laser used for optically trapping and moving target cells into a reaction chamber (Figure 8 (c)). A 1064nm pulsed laser was used to generate micro bubbles lysing the cells (Figure 8d). Finally, a 473nm blue laser served as an excitation light source for total internal reflection fluorescence (TIRF) measurement for protein immunoassay (Figure 8 (e)). This work is the first to prove that three optically operated single-cell assay functions can be integrated in the microfluidic chip with a simplified design.

Lab on a Chip Accepted Manuscript

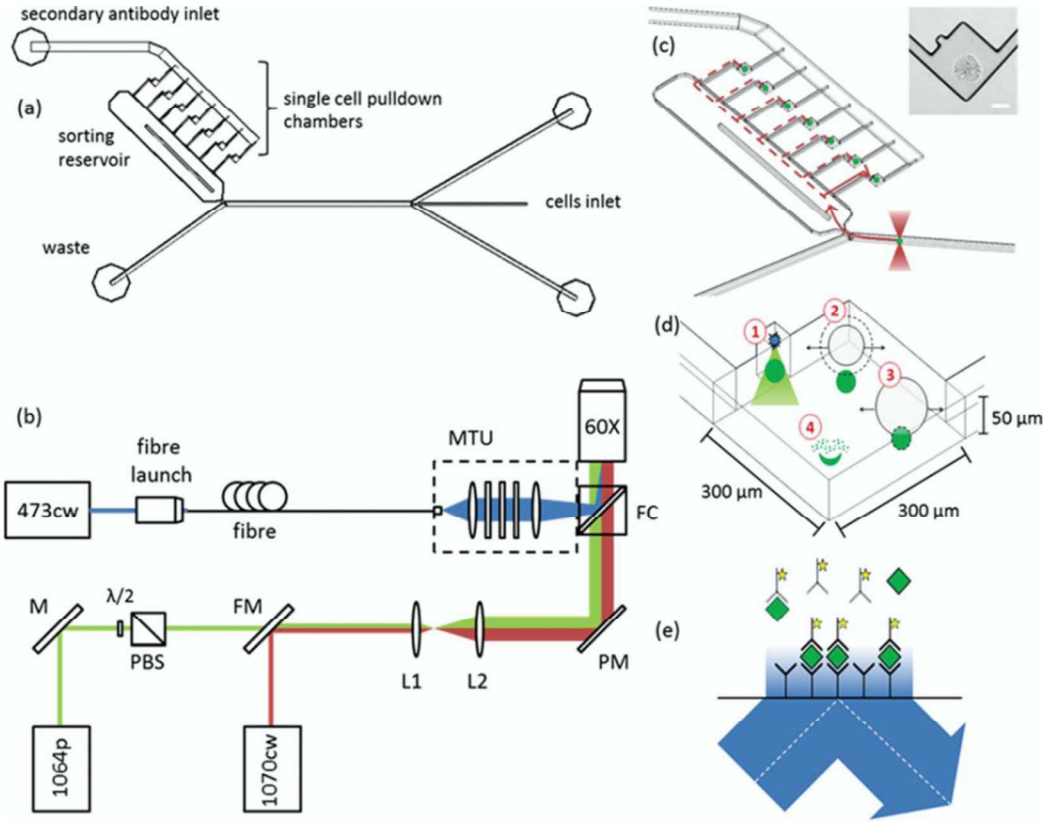

Figure 8 Schematic of "fully optical" single-cell analysis platform with functions of cell trapping, cell treatment, and intracellular protein detection. (a) On-chip microfluidic layout. (b) Off-chip optics setup (c) Optical trapping and manipulation of single cells to microfluidic reaction chambers. (d) Cell lysing with optically generated micro bubbles. (e) TIRF immunoassay to detect intracellular proteins.[104] Reproduction of the figures has been made with permissions from Royal Society of Chemistry.

We believe that FOM platforms have great potential in advancing future single-cell studies for the following reasons:

(1) Simplified microfluidic device architecture: No complicated microfluidic structure is needed to optically trap and sort cells with free-space optical components. Although optical alignment-free operations require integration of fiber-based optical waveguides in a microfluidic system, the fiber-optics integration is still easier than integration of electrical or mechanical components, which usually entails embedding of electrodes into a microfluidic network or precise design of channel

Lab on a Chip Accepted Manuscript

geometries enabling high-selectivity cell sorting. The simplified device design is the key to facilitate the integration of multiple cell-assay processes on a common chip platform.

(2) Fast cellular operation: a femtosecond pulsed laser operation enables very fast single cell treatment. The optical treatment process rapidly creates a small membrane pore for transfection or injection, which electrical or mechanical methods cannot achieve. It will hugely increase the transfection efficiency and the cell survival rate when a minimum operation period is maintained.

(3) Flexibility toward cell-type and application variations: FOM platforms easily accommodate variations in cellular size, surface profile, and functional behavior. In contrast, redesign of the microfluidic architecture would be needed according to a change in the size or other physical properties of targeted cells if the system employed hydrodynamic inertial flow for cell manipulation. Optical operation is highly suited for multiplexed single-cell analysis or assay involving detection of dynamic cellular processes.

We still find a few challenges facing effort to increase the utility of FOM platforms. For example, some detection technique such as Raman spectroscopy requires a long integration time to achieve a high signal-to-noise ratio, thus limiting the overall throughput. Besides, the sample volume needs to be further reduced to improve the transfection/injection efficiency. Future research effort needs to focus on improving cell treatment throughput, injection efficiency, pre/post cell sorting accuracy, and integrability of multiple functionalities. For example, any advances in nanoparticle injection could have great impact on SERS, Raman, and fluorescence spectroscopy techniques for single-cell analysis.

We see some niche applications of optically operated optofluidics platforms. What if researchers need to study the surface profile or genotyping of rare cells, such as circulating tumor cells (CTC)? An optofluidic platform could first allow them to optically sort out the target cells into a treatment region. Then, they could optically transfect DNA or RNA into a cell with minimum damage using the same device to study the genotype of target cells. Finally, the phenotype of the cells could be studied by taking their fluorescent images or Raman spectra. Another potential application is sequential operation of optical gold nanoparticle injection into a single rare cell and SERS of the cell. With a much stronger signal intensity enhanced by the plasmonic effect, one could obtain a Raman spectral signature of the cell with a shorter integration time and minimum cellular photodamages.

Lab on a Chip Accepted Manuscript

Ongoing advances in photonics technology may lead future simplification and miniaturization of a setup for operating single-cell analysis FOM platforms. For example, lasers with multiple wavelengths are rapidly developing these days. In the near future, multi-function single-cell assay may be realized by a single laser light source of adjustable power, frequency mode, and wavelength. It can hugely simplify the optical alignment and whole optical system size. Similarly, photodetectors, such as CCD or PMT, are also seeing technological advancements toward achieving a large field of view (FOV), high spatial resolution, and photo sensitivity. The high-performance photodetectors can also improve the throughput and sensitivity of FOM platforms. Of course, the individual optofluidic-based single-cell analysis modules discussed in this paper can also be incorporated with other platforms utilizing fluidic, electrical, magnetic, and mechanical mechanisms. Hybrid systems linking optofluidic-based single-cell analysis methods with other non-optical techniques may serve as a practical platform to address important biological problems prior to a full establishment of FOM technology.

5. Acknowledgements

The authors acknowledge support by National Taiwan University New Faculty Research Grant (N.-T. H.); the National Science Foundation under grants CBET- 0966723 (K.K.) and CEBET-1263889 (K.K.); 2013 Hongik University Research Fund (J.H.S)

Lab on a Chip Accepted Manuscript

Reference

1. H. Yin and D. Marshall, *Current Opinion in Biotechnology*, 2012, **23**, 110-119.
2. J. Zhu and W. E. Paul, *Cell Res*, 2009, **20**, 4-12.
3. R. M. Welsh and L. K. Selin, *Nat Rev Immunol*, 2002, **2**, 417-426.
4. G. M. Whitesides, *Nature*, 2006, **442**, 368-373.
5. C. W. Yung, J. Fiering, A. J. Mueller and D. E. Ingber, *Lab Chip*, 2009, **9**, 1171-1177.
6. M. M. Wang, E. Tu, D. E. Raymond, J. M. Yang, H. C. Zhang, N. Hagen, B. Dees, E. M. Mercer, A. H. Forster, I. Kariv, P. J. Marchand and W. F. Butler, *Nat Biotechnol*, 2005, **23**, 83-87.
7. M. Werner, F. Merenda, J. Piguet, R. P. Salathe and H. Vogel, *Lab Chip*, 2011, **11**, 2432-2439.
8. S. B. Kim, S. Y. Yoon, H. J. Sung and S. S. Kim, *Anal Chem*, 2008, **80**, 2628-2630.
9. F. Bragheri, P. Minzioni, R. M. Vazquez, N. Bellini, P. Paie, C. Mondello, R. Ramponi, I. Cristiani and R. Osellame, *Lab Chip*, 2012, **12**, 3779-3784.
10. K. H. Lee, K. S. Lee, J. H. Jung, C. B. Chang and H. J. Sung, *Appl Phys Lett*, 2013, **102**, 141911.
11. J. R. Kovac and J. Voldman, *Anal Chem*, 2007, **79**, 9321-9330.
12. Y. Chen, T.-H. Wu, Y.-C. Kung, M. A. Teitell and P.-Y. Chiou, *The Analyst*, 2013, **138**, 7308-7315.
13. P. Y. Chiou, A. T. Ohta and M. C. Wu, *Nature*, 2005, **436**, 370-372.
14. K. W. Huang, Y. C. Wu, J. A. Lee and P. Y. Chiou, *Lab Chip*, 2013, **13**, 3721-3727.
15. R. B. Horsch, J. E. Fry, N. L. Hoffmann, D. Eichholtz, S. G. Rogers and R. T. Fraley, *Science*, 1985, **227**, 1229-1231.
16. U. K. Tirlapur and K. Konig, *Nature*, 2002, **418**, 290-291.
17. Y. Li, R.-C. Pong, J. M. Bergelson, M. C. Hall, A. I. Sagalowsky, C.-P. Tseng, Z. Wang and J.-T. Hsieh, *Cancer Research*, 1999, **59**, 325-330.
18. J. Rojo, V. Díaz, J. M. de la Fuente, I. Segura, A. G. Barrientos, H. H. Riese, A. Bernad and S. Penadés, *ChemBioChem*, 2004, **5**, 291-297.
19. E. C. Cho, L. Au, Q. Zhang and Y. Xia, *Small*, 2010, **6**, 517-522.
20. S. Romano Di Peppe, A. Mangoni, G. Zambruno, G. Spinetti, G. Melillo, M. Napolitano and M. C. Capogrossi, *Gene therapy*, 2002, **9**, 1271-1277.
21. F. L. Graham and A. J. van der Eb, *Virology*, 1973, **52**, 456-467.
22. M. Jordan, A. Schallhorn and F. M. Wurm, *Nucleic Acids Research*, 1996, **24**, 596-601.
23. P. L. Felgner, T. R. Gadek, M. Holm, R. Roman, H. W. Chan, M. Wenz, J. P. Northrop, G. M. Ringold and M. Danielsen, *Proceedings of the National Academy of Sciences*, 1987, **84**, 7413-7417.
24. J.-P. Behr, *Bioconjugate Chemistry*, 1994, **5**, 382-389.
25. X. Gao and L. Huang, *Gene therapy*, 1995, **2**, 710-722.
26. J. H. Felgner, R. Kumar, C. N. Sridhar, C. J. Wheeler, Y. J. Tsai, R. Border, P. Ramsey, M. Martin and P. L. Felgner, *Journal of Biological Chemistry*, 1994, **269**, 2550-2561.
27. M. A. Kay, D. Liu and P. M. Hoogerbrugge, *Proceedings of the National Academy of Sciences*, 1997, **94**, 12744-12746.
28. S. Bacchetti and F. L. Graham, *Proceedings of the National Academy of Sciences*, 1977, **74**, 1590-1594.
29. U. Protzer, M. Nassal, P.-W. Chiang, M. Kirschfink and H. Schaller, *Proceedings of the National Academy of Sciences*, 1999, **96**, 10818-10823.
30. L. Naldini, U. Blömer, F. H. Gage, D. Trono and I. M. Verma, *Proceedings of the National Academy of Sciences*, 1996, **93**, 11382-11388.
31. S. Durand, X.-N. Nguyen, J. Turpin, S. Cordeil, N. Nazaret, S. Croze, R. Mahieux, J. Lachuer, C. Legras-Lachuer and A. Cimarelli, *Journal of Virology*, 2013, **87**, 234-242.
32. G. Lambert, E. Fattal and P. Couvreur, *Advanced Drug Delivery Reviews*, 2001, **47**, 99-112.

Lab on a Chip Accepted Manuscript

33. V. Vijayanathan, T. Thomas and T. J. Thomas, *Biochemistry*, 2002, **41**, 14085-14094.
34. L. H. Rome and V. A. Kickhoefer, *ACS Nano*, 2012, **7**, 889-902.
35. K. Kitamura, B. Judkewitz, M. Kano, W. Denk and M. Hausser, *Nature Methods*, 2008, **5**, 61-67.
36. F. Barnabe-Heider, K. Meletis, M. Eriksson, O. Bergmann, H. Sabelstrom, M. A. Harvey, H. Mikkers and J. Frisen, *Nature Methods*, 2008, **5**, 189-196.
37. M. Cemazar, M. Golzio, G. Sersa, M. P. Rols and J. Teissie, *Current Pharmaceutical Design*, 2006, **12**, 3817-3825.
38. C. Favard, D. S. Dean and M.-P. Rols, *Current Gene Therapy*, 2007, **7**, 67-77.
39. S. Bao, B. D. Thrall and D. L. Miller, *Ultrasound in Medicine & Biology*, 1997, **23**, 953-959.
40. P. Marmottant and S. Hilgenfeldt, *Nature*, 2003, **423**, 153-156.
41. P. Prentice, A. Cuschieri, K. Dholakia, M. Prausnitz and P. Campbell, *Nature Physics*, 2005, **1**, 107-110.
42. E. F. Fynan, R. G. Webster, D. H. Fuller, J. R. Haynes, J. C. Santoro and H. L. Robinson, *Proceedings of the National Academy of Sciences*, 1993, **90**, 11478-11482.
43. D. M. Feltquate, S. Heaney, R. G. Webster and H. L. Robinson, *Journal of Immunology*, 1997, **158**, 2278-2284.
44. A. Porgador, K. R. Irvine, A. Iwasaki, B. H. Barber, N. P. Restifo and R. N. Germain, *Journal of Experimental Medicine*, 1998, **188**, 1075-1082.
45. S. A. Susin, H. K. Lorenzo, N. Zamzami, I. Marzo, B. E. Snow, G. M. Brothers, J. Mangion, E. Jacotot, P. Costantini, M. Loeffler, N. Larochette, D. R. Goodlett, R. Aebersold, D. P. Siderovski, J. M. Penninger and G. Kroemer, *Nature*, 1999, **397**, 441-446.
46. C. C. Mello, J. M. Kramer, D. Stinchcomb and V. Ambros, *Embo Journal*, 1991, **10**, 3959-3970.
47. Y. Zhang and L. C. Yu, *Current Opinion in Biotechnology*, 2008, **19**, 506-510.
48. Y. Hosokawa, S. Iguchi, R. Yasukuni, Y. Hiraki, C. Shukunami and H. Masuhara, *Applied Surface Science*, 2009, **255**, 9880-9884.
49. P. Mthunzi, K. Dholakia and F. Gunn-Moore, *Journal of Biomedical Optics*, 2010, **15**, 041507.
50. M. Tsukakoshi, S. Kurata, Y. Nomiya, Y. Ikawa and T. Kasuya, *Appl. Phys. B*, 1984, **35**, 135-140.
51. G. Palumbo, M. Caruso, E. Crescenzi, M. F. Tecce, G. Roberti and A. Colasanti, *Journal of Photochemistry and Photobiology B*, 1996, **36**, 41-46.
52. Y. Hosokawa, H. Ochi, T. Iino, A. Hiraoka and M. Tanaka, *Plos One*, 2011, **6**, e27677.
53. K. Kuetemeyer, J. Baumgart, H. Lubatschowski and A. Heisterkamp, *Appl. Phys. B*, 2009, **97**, 695-699.
54. C. T. A. Brown, D. J. Stevenson, X. Tsampoula, C. McDougall, A. A. Lagatsky, W. Sibbett, F. J. Gunn-Moore and K. Dholakia, *Journal of Biophotonics*, 2008, **1**, 183-199.
55. R. Lévy, U. Shaheen, Y. Cesbron and V. Sée, *Nano Reviews*, 2010, **1**, 4889.
56. C. McDougall, D. J. Stevenson, C. T. A. Brown, F. Gunn-Moore and K. Dholakia, *Journal of Biophotonics*, 2009, **2**, 736-743.
57. R. F. Marchington, Y. Arita, X. Tsampoula, F. J. Gunn-Moore and K. Dholakia, *Biomedical Optics Express*, 2010, **1**, 527-536.
58. H. A. Rendall, R. F. Marchington, B. B. Praveen, G. Bergmann, Y. Arita, A. Heisterkamp, F. J. Gunn-Moore and K. Dholakia, *Lab Chip*, 2012, **12**, 4816-4820.
59. P. Khanna, N. Ramachandran, J. Yang, J. Wang, A. Kumar, M. Jaroszeski and S. Bhansali, *Diamond and Related Materials*, 2009, **18**, 606-610.
60. C. X. Xu and X. F. Yin, *Journal of chromatography. A*, 2011, **1218**, 726-732.
61. C. Ke, A.-M. Kelleher, H. Berney, M. Sheehan and A. Mathewson, *Sensors and Actuators B: Chemical*, 2007, **120**, 538-544.
62. Y. Sasuga, T. Iwasawa, K. Terada, Y. Oe, H. Sorimachi, O. Ohara and Y. Harada, *Anal Chem*, 2008, **80**, 9141-9149.
63. B. Huang, H. Wu, D. Bhaya, A. Grossman, S. Granier, B. K. Kobilka and R. N. Zare, *Science*, 2007, **315**, 81-84.

Lab on a Chip Accepted Manuscript

64. N. Bao, G. C. Kodippili, K. M. Giger, V. M. Fowler, P. S. Low and C. Lu, *Lab Chip*, 2011, **11**, 3053-3056.
65. P. A. Quinto-Su, H.-H. Lai, H. H. Yoon, C. E. Sims, N. L. Allbritton and V. Venugopalan, *Lab Chip*, 2008, **8**, 408-414.
66. K. S. Phillips, H. H. Lai, E. Johnson, C. E. Sims and N. L. Allbritton, *Lab Chip*, 2011, **11**, 1333-1341.
67. R. N. Zare and S. Kim, *Annual Review of Biomedical Engineering*, 2010, **12**, 187-201.
68. F. S. O. Fritzsch, C. Dusny, O. Frick and A. Schmid, *Annual Review of Chemical and Biomolecular Engineering*, 2012, **3**, 129-155.
69. J. Guck, S. Schinkinger, B. Lincoln, F. Wottawah, S. Ebert, M. Romeyke, D. Lenz, H. M. Erickson, R. Ananthakrishnan, D. Mitchell, J. Käs, S. Ulvick and C. Bilby, *Biophysical journal*, 2005, **88**, 3689-3698.
70. S. Suresh, *Acta biomaterialia*, 2007, **3**, 413-438.
71. M. Diez-Silva, M. Dao, J. Han, C. T. Lim and S. Suresh, *MRS bulletin / Materials Research Society*, 2010, **35**, 382-388.
72. E. C. Faria, N. Ma, E. Gazi, P. Gardner, M. Brown, N. W. Clarke and R. D. Snook, *The Analyst*, 2008, **133**, 1498-1500.
73. Q. S. Li, G. Y. Lee, C. N. Ong and C. T. Lim, *Biochemical and biophysical research communications*, 2008, **374**, 609-613.
74. H. Morgan, T. Sun, D. Holmes, S. Gawad and N. G. Green, *Journal of Physics D: Applied Physics*, 2007, **40**, 61.
75. J. Emmelkamp, F. Wolbers, H. Andersson, R. S. Dacosta, B. C. Wilson, I. Vermes and A. van den Berg, *Electrophoresis*, 2004, **25**, 3740-3745.
76. J. Reed, J. Chun, T. A. Zangle, S. Kalim, J. S. Hong, S. E. Pefley, X. Zheng, J. K. Gimzewski and M. A. Teitell, *Biophysical journal*, 2011, **101**, 1025-1031.
77. S. Fore, J. Chan, D. Taylor and T. Huser, *Journal of optics*, 2011, **13**, 44021.
78. A. S. Stender, K. Marchuk, C. Liu, S. Sander, M. W. Meyer, E. A. Smith, B. Neupane, G. Wang, J. Li, J. X. Cheng, B. Huang and N. Fang, *Chemical reviews*, 2013, **113**, 2469-2527.
79. M. Wojdyla, S. Raj and D. Petrov, *J Biomed Opt*, 2012, **17**, 97006-97001.
80. Y. C. Tung, N. T. Huang, B. R. Oh, B. Patra, C. C. Pan, T. Qiu, P. K. Chu, W. Zhang and K. Kurabayashi, *Lab Chip*, 2012, **12**, 3552-3565.
81. F. Lautenschlager, S. Paschke, S. Schinkinger, A. Bruel, M. Beil and J. Guck, *Proc Natl Acad Sci U S A*, 2009, **106**, 15696-15701.
82. C.-W. Lai, S.-K. Hsiung, C.-L. Yeh, A. Chiou and G.-B. Lee, *Sensors and Actuators B: Chemical*, 2008, **135**, 388-397.
83. Y. Zheng, J. Nguyen, Y. Wei and Y. Sun, *Lab Chip*, 2013, **13**, 2464-2483.
84. Z. Yi and S. Yu, *Micro & Nano Letters, IET*, 2011, **6**, 327-331.
85. N. Bellini, F. Bragheri, K. C. Vishnubhatla, L. Ferrara, P. Minzioni, G. Cerullo, R. Ramponi, I. Cristiani and R. Osellame, *Proc. SPIE 7589, Frontiers in Ultrafast Optics: Biomedical, Scientific, and Industrial Applications X*, 2010, **7589**, 75890D.
86. I. Sraj, R. Jimenez, E. Hoover, J. Chichester, D. W. M. Marr, J. Squier and C. D. Eggleton, *Journal of Biomedical Optics*, 2010, **15**, 047010-047017.
87. W. G. Lee, H. Bang, H. Yun, J. Lee, J. Park, J. K. Kim, S. Chung, K. Cho, C. Chung, D. C. Han and J. K. Chang, *Lab Chip*, 2007, **7**, 516-519.
88. H. Yun, K. Kim and W. G. Lee, *Biofabrication*, 2013, **5**, 022001.
89. T. W. Remmerbach, F. Wottawah, J. Dietrich, B. Lincoln, C. Wittekind and J. Guck, *Cancer Res*, 2009, **69**, 1728-1732.
90. J. F. Ojeda, C. Xie, Y. Q. Li, F. E. Bertrand, J. Wiley and T. J. McConnell, *Opt Express*, 2006, **14**, 5385-5393.
91. D. P. Cherney and J. M. Harris, *Annual review of analytical chemistry*, 2010, **3**, 277-297.
92. D. V. Petrov, *Journal of Optics A: Pure and Applied Optics*, 2007, **9**, S139.
93. A. Bankapur, E. Zachariah, S. Chidangil, M. Valiathan and D. Mathur, *PLoS One*, 2010, **5**, e10427.

Lab on a Chip Accepted Manuscript

94. G. P. Singh, C. M. Creely, G. Volpe, H. Grotsch and D. Petrov, *Anal Chem*, 2005, **77**, 2564-2568.
95. U. Neugebauer, T. Bocklitz, J. H. Clement, C. Krafft and J. Popp, *The Analyst*, 2010, **135**, 3178-3182.
96. S. Dochow, C. Krafft, U. Neugebauer, T. Bocklitz, T. Henkel, G. Mayer, J. Albert and J. Popp, *Lab Chip*, 2011, **11**, 1484-1490.
97. K. Chen, Y. Qin, F. Zheng, M. Sun and D. Shi, *Opt. Lett.*, 2006, **31**, 2015-2017.
98. F. Zheng, Y. Qin and K. Chen, *J Biomed Opt*, 2007, **12**, 034002.
99. K. E. Hamden, B. A. Bryan, P. W. Ford, C. Xie, Y. Q. Li and S. M. Akula, *Journal of virological methods*, 2005, **129**, 145-151.
100. K. Ramser, W. Wenseleers, S. Dewilde, S. V. Doorslaer and L. Moens, *Spectroscopy*, 2008, **22**, 287-295.
101. R. D. Snook, T. J. Harvey, E. Correia Faria and P. Gardner, *Integrative Biology*, 2009, **1**, 43-52.
102. K. Ramser, J. Enger, M. Goksor, D. Hanstorp, K. Logg and M. Kall, *Lab Chip*, 2005, **5**, 431-436.
103. T. H. Wu, Y. Chen, S. Y. Park, J. Hong, T. Teslaa, J. F. Zhong, D. Di Carlo, M. A. Teitell and P. Y. Chiou, *Lab Chip*, 2012, **12**, 1378-1383.
104. A. Salehi-Reyhani, J. Kaplinsky, E. Burgin, M. Novakova, A. J. deMello, R. H. Templer, P. Parker, M. A. A. Neil, O. Ces, P. French, K. R. Willison and D. Klug, *Lab Chip*, 2011, **11**, 1256-1261.

Lab on a Chip Accepted Manuscript