Biosensors and Bioelectronics 68 (2015) 529–535

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Controllably moving individual living cell in an array by modulating signal phase difference based on dielectrophoresis

Xiaoliang Guo, Rong Zhu *

State Key Laboratory of Precision Measurement Technology and Instruments, Department of Precision Instrument, Tsinghua University, Beijing, China

ARTICLE INFO

Article history:
Received 29 December 2014
Received in revised form
21 January 2015
Accepted 22 January 2015
Available online 22 January 2015

Keywords:
Cell array manipulation
Dielectrophoresis (DEP)
Signal phase difference
In-situ cell measurement

ABSTRACT

This paper reports a novel dielectrophoresis (DEP) based method for manipulating individual living cells by modulating phase difference of electrical signals applied on DEP electrodes. A novel microchip with an array structure is also proposed, consisting of a plurality of quadrupole-electrode units patterned into array on a glass substrate with a pair of center electrodes locating at the center of each quadrupole-electrode unit. Living cells can be trapped and positioned at the center of each quadrupole-electrode unit by using negative DEP (nDEP) manipulation and form an array. The trapped cells in the array can be controllably moved from one position to another and even from one of quadrupole-electrode units to adjacent unit by changing the phase difference of the signals applied on the two pairs of opposite electrodes in each quadrupole-electrode unit. The microchip allows an efficient and flexible manipulation of individual living cells that can be applied to study single cells. The experiments are performed to verify that different types of cells (MCF-7 cell and HeLa cell) can be effectively distinguished between each other using the method without label and fluorometric measurements. An identification of individual living cell from dead cells is also well demonstrated.

1. Introduction

The analysis and monitoring of individual cell-to-cell interactions and the capability of individually controlling single cells and particles is of interest in various areas of the life sciences ranging from immunotherapy and cancer research to rare cell identification and isolation (Bocchi et al., 2009; Johnson-Chavarria et al., 2011; Lin et al., 2013). Studying individual cells to understand and appreciate small but significant differences in a similar population is often necessary (Lim et al., 2014). It is also needed to analyze a number of cells to get a statistical assessment, which can be realized by using an array structure that controls numerous cells simultaneously in one chip. Studies on single cell, cell array for statistics, and efficient control for manipulating individual cells in an array have a great mean.

Many methods have been reported for manipulating micro/nano particles or cells. Optical tweezers (OT) use a highly focused laser beam to trap and manipulate microscopic dielectric particles with highly free and precise way. Single cell manipulation can be realized using OT (Cheah et al., 2014; Moffitt et al., 2008; Roy et al., 2014). Optical dielectrophoresis (ODEP) is another approach to realize a flexible manipulation of particles and cells (Huang et al., 2012, 2013; Liang et al., 2012). Despite impressive performances of such optics-based manipulating techniques, they generally require bulky, complicated optical setups, which are difficult to be miniaturized. Acoustic tweezers (Courtney et al., 2014; Shi et al., 2009) utilize standing surface acoustic wave (SSAW) to manipulate and pattern cells or particles. This technique is capable of patterning cells and particles regardless of their shapes, sizes, charges or polarities and with low power-consumption.

Dielectrophoresis (DEP) is another one of the most versatile methods for particle manipulation due to its label-free nature, favorable scaling effects, simple device and capability to integrate with in-situ cell measurements. DEP is a translational motion of a particle or cell by induced polarization in a non-uniform electric field (Li, 2008). DEP-based manipulation is an effective method to move and trap particles or cells (Jubery et al., 2014; Sankaran et al., 2008), and it is amenable for miniaturization of devices. The majority of dielectrophoretic manipulation includes translating (dielectrophoresis), rotating (electrorotation), orienting (electro-orientation), trapping and using traveling wave dielectrophoresis (Li, 2008). So far many electrode structures used for DEP-based manipulation have been reported: such as quadrupole electrodes (Frenea et al., 2003), annular electrodes (Mittal et al., 2007), 3D-electrodes (Iliescu et al., 2007), as well as insulating electrophoresis (Lapizco-Encinas et al., 2004). The non-uniform electric field can be produced with an arrangement of conductive electrodes or insulating posts in a micro-channel with remote electrodes

* Corresponding author.
E-mail address: rong_zhu@263.net (R. Zhu).

http://dx.doi.org/10.1016/j.bios.2015.01.052
0956-5663/

(Lapizco-Encinas et al., 2004). Modulating phases of signals applied on electrodes is another way to control the motions of particles or cells (Wang et al., 2013). Particle rotation (electrorotation, ROT) and particle conveyance (traveling wave dielectrophoresis, TWD) based on phase modulation have been reported. Particle rotation is realized by applying signals with phase differences onto encircled electrodes (quadrupole-electrode or 3D octode (Han et al., 2013)). Traveling wave dielectrophoresis is a linear application of electrorotation, impelling particle to a linear conveyance along a series of electrodes (Cen et al., 2004). Even though there have been great progresses on DEP-based manipulation, controllably positioning individual living cells into array and moving the cells in the array for executing in-situ cell measurement are still challenging.

In this paper, we propose an advanced method to manipulate and position individual cells into an array with nDEP, and move the trapped cells flexibly by dynamically modulating signal phase difference. A novel microchip is also proposed, consisting of a plurality of quadrupole-electrode units patterned into array (4-by-8 grid) on a glass substrate with a pair of center electrodes locating at the center of each quadrupole-electrode unit. Compared with other DEP-based methods, the developed microchip and manipulation method can realize efficiently positioning individual living cells into an array and controllably moving the cells from one position to another in several seconds by using nDEP with phase difference modulation. For the first time, this manipulation method is employed to experimentally distinguish single cell between MCF-7 cell and HeLa cell using the travel time of the cell moving from one specific position to another under nDEP manipulation with different frequency and different amplitude of voltage. The velocity of the cell implies the DEP forces imposed on the cell, which embodies the physiological properties of the cell. An array with a plurality of quadrupole-electrode units ensures a statistical assessment on the cells. The method is also applied to identify individual living cells from dead cells. The phase difference modulation method is easily operated and applicable for diverse single cell studies without label and fluorometric measurements.

2. Materials and methods

2.1. Theory and simulation

The time-averaged DEP force can be formulated as (Pohl, 1951)

$$F_{DEP} = 2\pi R^3 \varepsilon_m \,\mathrm{Re}\,(f_{CM})\nabla E^2 \tag{1}$$

where R is the particle or cell radius, ε_m is the permittivity of the suspension medium, E is electric field intensity, ∇ is the is the del vector operator, f_{CM} is the Clausius–Mossotti (CM) factor (Pohl, 1951):

$$f_{CM} = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \tag{2}$$

where ε_p^* and ε_m^* is the complex permittivity of the particle or cell and the suspension medium respectively. The complex electric permittivity can be represented as

$$\varepsilon^* = \varepsilon - j\frac{\sigma}{\omega} \tag{3}$$

where $j = \sqrt{-1}$, σ is the electric conductivity, ω is the electric field angle frequency.

The real part of the factor f_{CM} is a function of the electric field frequency depending on the dielectric properties of the medium and particles or cells, and can be either positive or negative. If the real part of f_{CM} is positive, the particle or cell is attracted to a region with high electric field, called positive DEP (pDEP), and if the real part of f_{CM} is negative, the particle or cell experiences a negative DEP (nDEP) and is impelled toward a region with low electric field. The nDEP manipulation ensures a good viability of cells, and thus be used in this study.

A living cell is generally modeled as a single-shell dielectric model, and the f_{CM} is formulated as (Jones, 2005)

$$f_{CM} = \frac{\omega^2(\tau_m\tau_c^* - \tau_c\tau_m^*) - 1 + j\omega(\tau_m^* - \tau_m - \tau_c^*)}{2 - \omega^2(2\tau_m\tau_c^* + \tau_c\tau_m^*) + j\omega(\tau_m^* + 2\tau_m + \tau_c^*)} \tag{4}$$

where $\tau_c^* = C_m R_c/\sigma_c$, $\tau_c = \varepsilon_c/\sigma_c$, $\tau_m^* = C_m R_c/\sigma_m$, $\tau_m = \varepsilon_m/\sigma_m$, R_c is the cell radius, C_m is the specific cell membrane capacitance, σ_c is the conductivity of the cell, σ_m is the conductivity of the suspending medium, ε_c is the permittivity of the cell and ε_m is permittivity of the suspending medium.

Exerting nDEP forces on cells suspending in culture medium will impel the cells toward the region with the minimal electric field intensity. A unit of quadrupole circular electrodes with a pair of center electrodes is shown in Fig. 1(A) and the electrode array layout of the microchip is shown in Fig. 1(B). Two alternating current (AC) signals with the same frequency and same amplitude but with a phase difference of θ were applied to the two pairs of opposite circular electrodes in each quadrupole-electrode unit whilst the center electrodes were grounded. Distributions of the electric field intensity under different phase difference were simulated using COMSOL. We established a model with a diameter of 2.5 mm and a height of 100 μm to simulate the electric field distribution on the microchip with an electrode array of 4-by-8 grid as shown in Fig. 1(B). The material of the model was set as a culture medium and the boundary was set as an insulator. Electric current module and electrical circuit module were used in simulation, and above 600,000 elements were meshed. The simulation results of the electric field distribution in one quadrupole-electrode unit are shown in Fig. 1(C)–(G). We found the region with the minimal electric field intensity in the unit could be changed by varying the phase difference θ. When the phase difference was set to be 180°, the minimal electric field intensity located at the center of the quadrupole unit as shown in Fig. 1(C). When the phase difference was gradually varied from 180° to 0° as shown in Fig. 1 (C)–(G) (Fig. 1(C) 180°, Fig. 1 (D) 135°, Fig. 1(E) 90°, Fig. 1(F) 45°, and Fig. 1(G) 0°), the region with the minimal electric field intensity moved from the center of the unit to the boundary. This indicates that the cell can be trapped and moved controllably from the center to the boundary by modulating the phase difference of nDEP.

As mentioned before, the factor f_{CM} depends on the size and the dielectric properties of cells. Different cell has different physiological properties, and thus generates a different DEP force exerted on the cell in a same electric field that makes the cell moving at different rate. The movement efficiency of the cell implies the physiological property of the cell.

2.2. Fabrication and structure

A complete structure of the microchip is shown in Fig. 2. The microchip has a plurality of quadrupole-circular-electrode units formed an array (4-by-8 grid) and at the center of each quadrupole-electrode unit there locates a pair of center electrodes. The pairs of center electrodes can be used to perform in-situ measurements on the cell that has been trapped onto the electrodes. In this study, we focused on the cell manipulation control. In order to integrate an array structure onto one chip, a smart crossed interdigital bus-bar network connecting the electrodes to the contact-pads as shown in Fig. 2(C) was proposed. The network consists of