

Microfluidic chemical cytometry based on modulation of local field strength†

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A simple microfluidic device was demonstrated to analyze intracellular contents from single cells with high throughput based on having different field strengths in geometrically defined sections of a microchannel for electrical lysis and electrophoresis.

Rapid chemical analysis of intracellular materials from single cells is of considerable interests to chemical, biological and pharmaceutical researchers. The analysis of intracellular proteins using conventional flow cytometry remains difficult.^{1,2} In flow cytometry, antibodies are often used for staining specific proteins in the cells. Antibodies could be trapped in the cytoplasm to create a nonspecific background. More seriously, the typical procedure of fixing and permeabilizing cells can often modify or destroy some antigens that are being assayed. In comparison, the newly emerged chemical cytometry allows molecular analysis of individual cells with revelation of the heterogeneous nature of the cell population.^{3–8} It typically requires the disruption of cell membrane and the separation and detection of intracellular contents (mostly proteins) using high sensitivity methods such as electrophoresis coupled with laser-induced fluorescence.⁹ The approach has shown excellent potential in determining the cell-to-cell distribution of protein expression within a cell population without the cumbersome procedure of fixing and permeabilizing.

Microfluidics has been demonstrated as a new platform for chemical cytometry recently.^{10–13} Throughput as high as 7–12 cells min⁻¹ was demonstrated.¹⁰ High throughput allows the analysis of cell populations of considerable sizes to generate the results with statistical significance. In this communication, we demonstrate a simple microfluidic device which carries out chemical analysis of single cells at a maximum rate of 75–85 cells min⁻¹ operated with a single DC power supply and a syringe pump. The fields for electrical lysis of cells and electrophoresis of intracellular materials are designed to have different intensities, determined by the local geometry of the channel. The confinement of the high field for cell lysis in a small section of the device alleviates the adverse effects from Joule heating and decreases the overall voltage needed for the operation.^{14,15}

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The microfluidic device was fabricated on PDMS using standard soft lithography (the details of the microfabrication and operation of the device are included in the ESI†).¹⁶ We apply electrical lysis to disrupt the membrane rapidly.^{10,17} A field intensity of 1000–1500 V cm⁻¹ or higher is typically needed if we want the cell membrane to rupture within tens of milliseconds. This field intensity would generate excessive Joule heating if used for electrophoresis, which could undermine the separation efficiency and affect biological activities of the species being assayed. The design of our device is shown in Fig. 1. The system consisted of two cross channels perpendicular to each other. The width of the horizontal channel altered across its length in different sections (labeled as 1, 2 and 3 in Fig. 1(b)). Sections 2 and 3 in Fig. 1(b) with different widths (40 and 70 μm, respectively) in the horizontal channel were specifically used for electrical lysis of cells and electrophoretic separation of intracellular contents, respectively. As shown in previous work from the literature and our group, the local electric field intensity in a microfluidic channel was inversely proportional to the local width when the depth of the channel was uniform, due to Ohm's law.^{14,15,18,19} When a dc field is established in the horizontal channel, we have $E_2/E_1 = W_1/W_2 = 7.25$ and $E_2/E_3 = W_3/W_2 = 1.75$ where E_1 , E_2 , E_3 and W_1 , W_2 , W_3 are the field intensities and widths in sections 1, 2 and 3. For example, if we assume that the 100 μm area in between two 40 μm-wide channels has a field intensity close to that of E_2 , when we apply a total voltage of 3550 V across the horizontal channel, we obtain an E_2 of 1571 V cm⁻¹, E_1 was 217 V cm⁻¹, and E_3 was 898 V cm⁻¹. In this experiment, cells were lysed rapidly in section 2 when they were flowed in from inlet 1. The long separation channel with lower field E_3 was used for the subsequent electrophoretic separation of intracellular contents. This practice guaranteed that E_2 was high enough for rapid lysis whereas E_1 and E_3 were at much lower intensity to decrease overall Joule heating in the system. Two steady pressure-driven flows were created by a syringe pump from inlets 1 and 2. The flow from inlet 1 fed cells continuously into the system and the flow from inlet 2 gently pinched down the flowing cells close to the entrance of the separation channel. The two flows also helped to alleviate local hot spots.

We used Chinese hamster ovary cells (CHO-K1) as our model cells in all the experiments (details about cell culture and treatment are described in the ESI†). For observing the leak of intracellular materials during electrical lysis, cells were loaded with a fluorogenic dye, calcein AM (Molecular Probes, Eugene, OR) and the microchip was mounted on an inverted fluorescent microscope. The hydrolysis of calcein AM by intracellular esterases produces calcein, a hydrophilic, strongly fluorescent compound

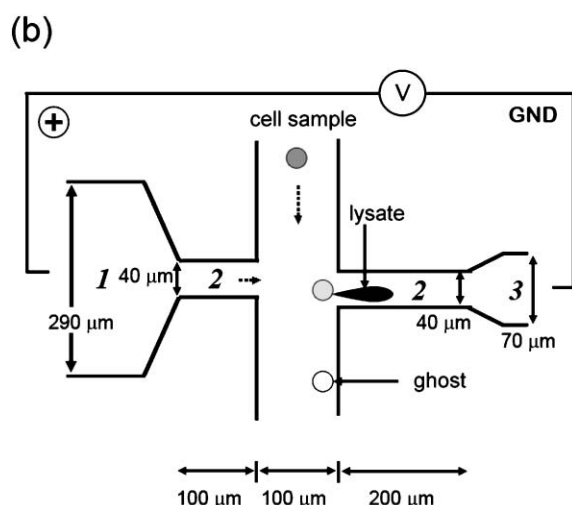
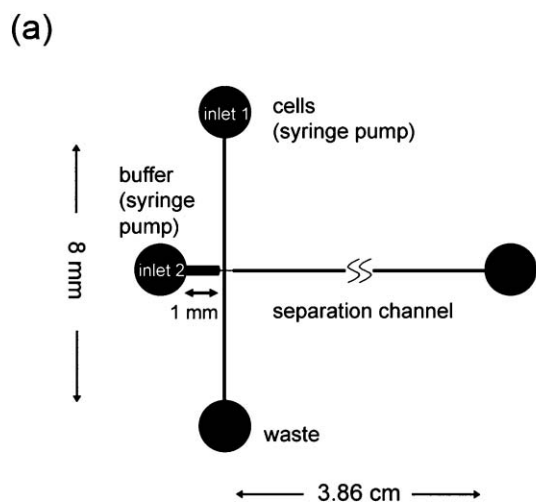


Fig. 1 (a) The layout of the microfluidic device for chemical cytometry (not to scale). The inlets 1 and 2 were connected to a syringe pump with the cell sample and the buffer fed in, respectively. (b) The scheme of cell lysis at the intersection. The broken line arrows show the pressure-driven flows. A DC electric voltage was established across the horizontal channel. The horizontal channel was divided in three sections (1, 2 and 3) based on their difference in the width. The two 40 μm channels were off by 20 μm so that the flow from inlet 2 could push the cells into a single file and get them close to the entrance of the separation channel.

(495/517 nm) that is well-retained in the cell cytoplasm. The cells had diameters of 10–17 μm . The pressure-driven flow from inlet 2 could effectively decrease the probability of having two cells lysed simultaneously. The vertical channel had a width about 100 μm to ensure that the channel did not get clogged easily by cells or cell ghosts. An isotonic buffer with low conductivity (10 mM phosphate and 250 mM sucrose, pH = 7.4) was applied in the experiments.

Fig. 2 shows the overlay images of the same cell at different temporal points and different physical locations while passing the lysis intersection. The cell was lysed in less than 30 ms (from frame 2 to 3) when it passed the field with E_2 at 1571 V cm^{-1} . The cell lysate entered the separation channel entirely, following the direction of the electric field. The cell ghost continued to flow in the vertical channel and entered a waste reservoir eventually. Based

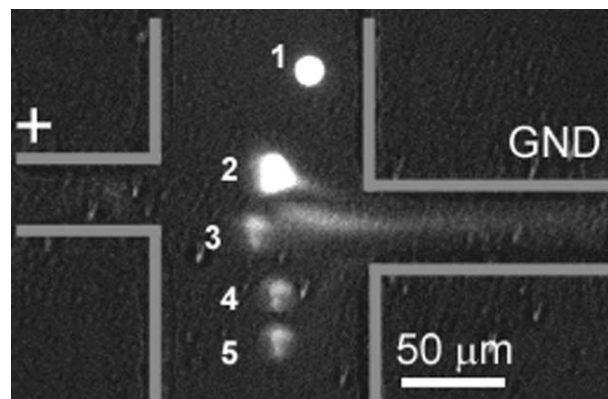


Fig. 2 The overlay image series of the same cell showing its electrical lysis. The pictures were taken at a frame rate of 33 Hz. The gray lines indicate the edges of channels; $E_2 = 1571 \text{ V cm}^{-1}$.

on the literature, the field strength at the intersection of the channel should be close to that of E_2 (1571 V cm^{-1}).^{20,21} The rapid cell lysis (100% cells lysed within 30 ms) also confirmed that the local field intensity was higher than 1200 V cm^{-1} based on our previous results.¹⁵ We found that the high intensity of E_2 was critical to realize high throughput. When E_2 was lower than 1500 V cm^{-1} and the throughput of cells was 30 cells min^{-1} or higher, the lysis happened less rapidly and there could be partially lysed cells entering and clogging the separation channel. The high field for lysis was essentially confined to a length of 400 μm in the horizontal channel. No bubbling was observed during 3–5 minutes of continuous operation each time. This agrees with previous reports that confining high fields (up to 100 kV cm^{-1}) in short lengths of microfluidic channels or capillary tubes did not result in boiling of the buffer.^{18,19} The pressure-driven flows might have also helped alleviate local Joule heating problem. As a comparison, we also tried a different layout of the device with the width of the section 3 at 40 μm and other dimensions the same. In that case, rapid electrical lysis (30–60 ms) could only be achieved with a much higher total voltage (around 5000 V). Under these conditions, PDMS around the separation channel turned opaque after 20–30 s of operation indicating boiling of the buffer inside. These results indicated that the specific geometry of the channel was important for lowering the electrophoresis field intensity (E_3) into a range which was suitable for free solution electrophoresis while confining ultrahigh field intensity desired by rapid cell lysis in a small section.

When the density of the cell suspension was around 10^7 cells ml^{-1} and the flow rates from inlets 1 and 2 were 20 and 10 $\mu\text{l h}^{-1}$, respectively, the maximum throughput achieved in the microfluidic system was around 75–85 cells min^{-1} with a E_2 of 1571 V cm^{-1} . Fig. 3 shows the fluorescence signal generated from lysed CHO cells at 200 μm into the separation channel under these conditions. The peaks were generated from calcein in the lysate of separate single cells and this is confirmed by comparing the sizes of these peaks with those generated by low concentration cells (as shown in Fig. 4). The peak area was similar for each individual cell with small variation. Some small peaks might have been generated by membrane fragments. This is about five-fold higher than the highest throughput reported so far, achieved using a more sophisticated microfluidic system.¹⁰ The electrophoresis of calcein

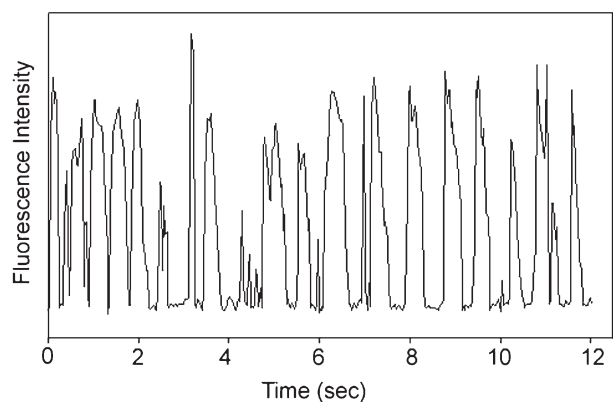


Fig. 3 Detection of calcein released from single cells (10^7 cells ml^{-1}) at around $200\ \mu\text{m}$ into the separation channel; E_2 was $1571\ \text{V cm}^{-1}$ and E_3 was $898\ \text{V cm}^{-1}$. The throughput was around $75\text{--}85$ cells min^{-1} .

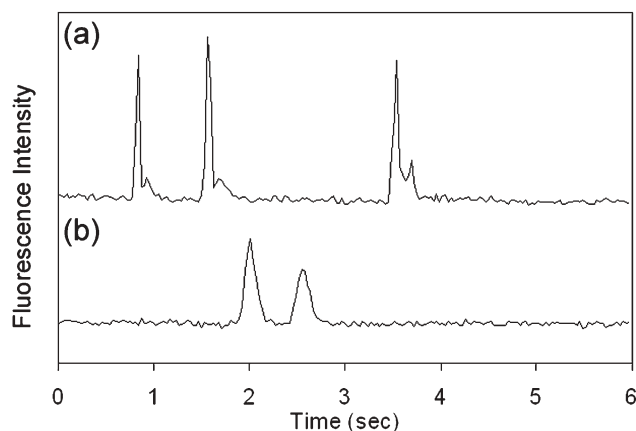


Fig. 4 Detection of calcein released from single cells (10^6 cells ml^{-1}) in the separation channel; E_2 was at $1571\ \text{V cm}^{-1}$ and E_3 was $898\ \text{V cm}^{-1}$. (a) At $200\ \mu\text{m}$ into the separation channel. (b) At $1\ \text{cm}$ into the separation channel.

which was in the cytoplasm was demonstrated here as a proof-of-concept for analysis of cell lysate from single cells. Fig. 4(a) (detected at $200\ \mu\text{m}$ into the separation channel) shows that when the throughput was low, the peaks from individual cells were clearly distinguishable from each other. Two peaks were observed from calcein. Since the change from calcein AM to calcein involves the generation of several carboxylic acid groups, the heterogeneity in the fluorescent calcein was the possible reason for multiple peaks. Fig. 4(b) shows that the second peak disappeared when the observation was made at $1\ \text{cm}$ into the separation channel. This is

possibly due to the adsorption of molecules on the untreated (only oxidized) PDMS surface or the stacking due to the field intensity decrease.

In this work, we created different field intensities for cell lysis and electrophoresis in the same microfluidic channel by designing the geometry of the local sections. This solves the issues with excessive Joule heating associated with the high field strength needed for cell lysis. Our device has the potential for carrying out studies related to single cell proteomics. The simplicity of the device will allow easy scale-up for parallel operations and integration with other devices. Further improvement can be made by modifying the PDMS surface to suppress the adsorption of biomolecules.

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