Transport of Biomolecules in the Ratcheting Electrophoresis Microchip (REM)*

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Ratcheting electrophoresis microchip (REM) is a novel concept of a microfluidic device proposed by the authors for the electrophoretic separation of macromolecules such as DNA and proteins in aqueous solution. In the present report, a new type of REM is proposed. The first prototype of the REM, which consists of a microchannel and an array of thousands of parallel linear microelectrodes with a width of $\sim 2\ \mu m$ and a pitch of $\sim 10\ \mu m$ embedded in the wall of the microchannel, has some problems: dispersion of analyte molecules is large when they leave the surface of the electrodes in the direction parallel to the surface, and the small width of the microelectrodes that are needed to minimize the dispersion of molecules makes the chip susceptible to the Debye screening. To solve these problems, the crosswise migration type is proposed here, where electrophoretic migration is driven as crossing the microchannel, which results in minimized dispersion of analyte molecules and effective electric field over the whole channel that is free from the Debye screening. Computational simulation has been performed and satisfactory results were obtained.

Key Words: Mass Transfer, Electrophoresis, Ratcheting, Microchip, Biomolecules

1. Introduction

The separation of biomolecules, such as DNA and proteins, is one of the most important techniques in biotechnology and there is an increasing need for more efficient methods. Electrophoresis has been predominantly used for that purpose for many years and a huge amount of data obtained by electrophoresis has been accumulated as academic resources. In the conventional slab gel electrophoresis, a uniform electric field of $\sim 10\ V/cm$ is applied on a slab of agarose or polyacrylamide gel and biomolecules are separated according to differences in the distance of their electrophoretic migration, i.e., mobility. Voltages in the order of 100 V are generally required, while the time for separation is at least 1 hour, often overnight. The time is markedly reduced by capillary electrophoresis[1], a sophisticated version of conventional electrophoresis. However, capillary electrophoresis still has problems such as the requirement of high voltage (typically 1 kV), size of the equipment used and lack of flexibility.

Recently, several microdevices have been proposed in the literature; these include thermal ratchet devices that use specially designed microchannels[2],[3] and saw-tooth electric fields[4],[5], devices with an array of hooking obstacles[6],[7], and entropic trap arrays[8]. However, these devices do not simultaneously possess three important features: resolution of separation, flexibility and speed. Although capillary electrophoresis with microfabricated channels[9],[10] has been progressing toward a "lab on a chip" by integrating some functions such as sample supply and detection of analyte molecules, it generally suffers from low separation efficiency mainly due to its short migration path[1].

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The authors have proposed a new microscale electrophoresis concept using a ratcheting mechanism by applying a saw-tooth electric field, which is generated by microelectrodes embedded in the wall of a microchannel filled with a sample solution[11]. The first prototype of the Ratcheting Electrophoresis Microchip (REM) has been designed and it is now in the process of fabrication and demonstration experiment in University of California, Berkekey[12].

The present report describes the transport of DNA molecules in the REM and discusses refinements which could be introduced toward realization of the modified device. A crosswise migration type where electrophoresis is driven as crossing the microchannel is proposed here.

Nomenclature

c : concentration
D : diffusion coefficient, m^2/s
E : electric field, V/m
k_B : the Boltzmann constant, J/K
L : length of DNA, base pairs
q : electric charge, C
T : temperature, K

Greek symbols

ε_0 : absolute electric permittivity, $C^2/(Nm^2)$
ε_r : dielectric constant
ϕ : electric potential, V
ω : electrophoretic mobility, $m^2/(Vs)$

2. Basic Mechanism of the REM

The ratcheting electrophoresis microchip (REM) consists of a microchannel and an array of thousands of parallel linear microelectrodes with a pitch of ~10 μm embedded in the wall of the microchannel. The controlled voltages of the microelectrodes generate an electric field in the aqueous solution in the channel, and realize microscale electrophoresis with the sorting cycle as shown in Fig. 1. In steps 1 and 2, the potential wells are moved to the left, which makes the mixture of analyte molecules move to the bottom of the well. During steps 3 and 4, the molecules are moved by localized electrophoresis between the electrodes. Electrophoresis is terminated by changing the voltage pattern to the one shown in step 5. The time period for steps 3 and 4 determines the threshold value of electrophoretic mobility for the sorting. The analyte molecules are sorted into two groups at this threshold and these two groups move in two different directions; molecules having mobilities larger than the threshold move forward while those having smaller mobilities move backward. The sieving cycle is completed only with a unit of four electrodes. Therefore, thousands of microelectrodes of the REM allow sieving at hundreds of electrophoresis units in parallel. Microelectrodes spatially arranged with a very small separation (~10 μm) produce high electric fields (~1 000 V/cm) between them by using low voltages (~1 V). The low voltage applied here is desirable to prevent electrolysis of water, and also for compatibility with modern electronics for voltage control, which is essential for a programmable device. Although the resolution of the electrophoresis by a single sorting cycle deteriorates because of its very short migration path (~10 μm), it can be compensated by the repeated use of the cycle. The REM can be produced by the standard microlithography technique and realizes some unique functions that conventional electrophoresis does not perform.

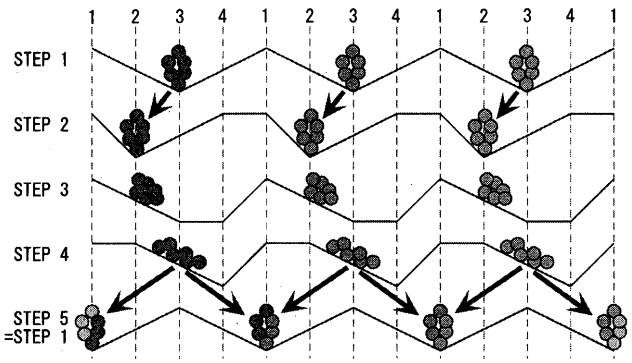


Fig. 1 Sorting cycle of the REM. Dashed lines indicate position of microelectrodes. Solid lines show electric potential produced in the solution by the microelectrodes.

3. Transport of DNA in the REM

3.1 Single array system

The authors have designed the first prototype of REM as shown in Fig. 2, which is named as the single array system. The characteristics of transport and separation of DNA in the microchannel of the chip were examined by a computational simulation. The details of the simulation method have been reported in Ref. (11) and hence the method is described here only briefly.

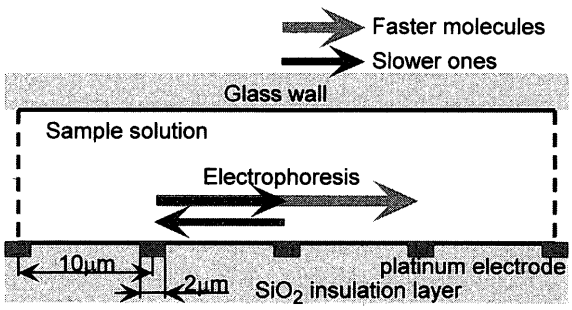


Fig. 2 Configuration of the single array system

The time-varying concentrations of DNA, H^+ and OH^- ions and the transient electric field in the solution were solved by the following governing equations. For the concentration of ions,

$$\frac{\partial c_i}{\partial t}+\frac{\partial c_i u_{i,x}}{\partial x}+\frac{\partial c_i u_{i,y}}{\partial y}=\frac{\partial}{\partial x}D_i\frac{\partial c_i}{\partial x}+\frac{\partial}{\partial y}D_i\frac{\partial c_i}{\partial y}, \tag{1}$$

where c denotes concentration of ions. Subscript i indicates ion species, i.e., DNA, H^+ and OH^-. The electrophoretic migration velocity u is given by

$$u_{i,x}=-\omega_i\frac{\partial\phi}{\partial x},\quad u_{i,y}=-\omega_i\frac{\partial\phi}{\partial y}, \tag{2}$$

where ϕ denotes electric potential. Diffusion coefficient D for DNA is given by

$$D=D_{50\,\mathrm{mer}}\left(\frac{L}{50}\right)^{-1/3}, \tag{3}$$

on the assumption that DNA molecules are spherical and its diffusion coefficient is inversely proportional to its radius. Here, L is the length (number of base pairs) of DNA. Mobility ω for DNA is given by the Einstein relation

$$\omega=\frac{Dq}{k_B T}, \tag{4}$$

where k_B is the Boltzmann constant. Temperature T is assumed to be 300 K. Electric charge q for DNA is assumed to be equal to $L\cdot e$ where e is the elementary electric charge.

For $H^+$and OH^-, mobility is given as 3.63×10^{-7} and -2.05×10^{-7} $m^2/(Vs)$, respectively[13]. Using these values with Eq.(4), diffusion coefficient is determined to be 9.38×10^{-9} and 5.30×10^{-9} m^2/s, respectively.

The diffusion coefficient and mobility for H^+, OH^- and all species of DNA that are considered as analytes are shown in Table 1.

The transient electric potential ϕ is determined by solving the following equation.

Table 1 Diffusion coefficient, D, electric charge, q, and mobility, ω, of H^+, OH^- and sample DNA. q is shown as a ratio to the elementary charge, $e=1.60\times10^{-19}$ C.

species	diffusion coefficient D (m^2/s)	electric charge q/e	mobility ω (m^2/Vs)
H^+	9.38×10^{-9}	1	3.63×10^{-7}
OH^-	5.30×10^{-9}	−1	-2.05×10^{-7}
DNA 35mer	2.03×10^{-11}	−35	-2.74×10^{-8}
DNA 45mer	1.86×10^{-11}	−45	-3.25×10^{-8}
DNA 50mer	1.80×10^{-11}	−50	-3.48×10^{-8}
DNA 55mer	1.74×10^{-11}	−55	-3.71×10^{-8}
DNA 65mer	1.65×10^{-11}	−65	-4.15×10^{-8}

$$\frac{\partial}{\partial x}\varepsilon_r\frac{\partial\phi}{\partial x}+\frac{\partial}{\partial y}\varepsilon_r\frac{\partial\phi}{\partial y}=-\frac{\sum_i q_i c_i}{\varepsilon_0}. \tag{5}$$

Here, $\varepsilon_0=8.8542\times10^{-12}$ $C^2/(Nm^2)$ and $\varepsilon_r=78.5$ are the absolute electric permittivity of vacuum and the dielectric constant of water, respectively. Species i correspond to H^+ and OH^-.

Equation (5) was solved by the finite difference method. On the other hand, it was rather difficult to solve the mass diffusion equation (Eq.(1)) by the finite difference method particularly for DNA because of its extremely small diffusion coefficient. Therefore, Eq.(1) was solved by the Langevin equation, which is compatible with Eq.(1).

An example of the simulation results[11] is shown in Fig. 3. A mixture of DNA 65 mer and 35 mer was initially captured at the second electrode from the left (step 2 of Fig. 1), which is followed by the electrophoresis stage (steps 3 and 4). The snapshot shown in Fig. 3 is taken at the end of step 4, where an obvious difference is observed between migrations of the two species of DNA molecules. Immediately after this, these molecules are sorted forward (65 mer) and backward (35 mer) at step 5. Thus, a series of simulations verified that excellent resolution of separation can be obtained by repeating the sorting cycle.

On the other hand, two problems were identified in the simulations. One is susceptibility to the Debye screening. Even with pure deionized water that contains only H^+ and OH^- at 10^{-7} M, the voltage of the electrodes is shielded by concentrated H^+ or OH^- ions if there is plenty of water around the electrodes; this leads to a failure of the transport function. It was found that the ratio of channel volume to surface area of electrodes should be less than 60 μm to prevent the Debye screening, which requires that the channel height should be less than 3 μm for the present configuration of the chip where electrodes have a width of 2 μm with a pitch of 10 μm. In the case of larger channel height, an electric field effective in driving analyte molecules is not formed all over the channel[11]. The other problem of the single array

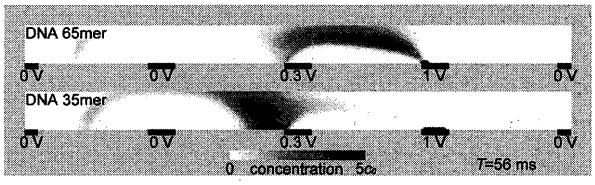


Fig. 3 Distribution of two species of DNA molecules to be separated, 65 mer (upper panel) and 35 mer (lower panel), in the microchannel at the end of the electrophoresis stage in the single array system with a channel height of 3 μm. Step 5 (see Fig. 1) follows just after this to divide these molecules into the forward and backward positions.

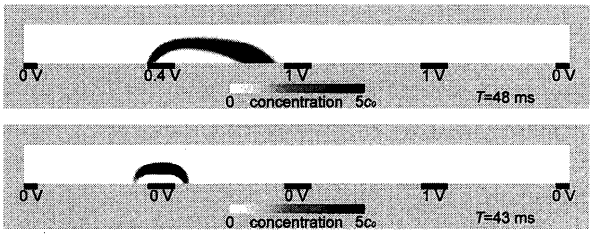


Fig. 4 Concentration distribution of analyte molecules (DNA 50 mer) at the start of electrophoretic migration. (a) Molecules are scattered with a wide dispersion when the electric field along the channel is applied to the molecules that are sticking to the electrode (upper figure). (b) "Launch" of molecules in the single electrode array system (lower figure).

system lies in the commencement of the electrophoresis stage. At the beginning of step 3 in Fig. 1, we expect that analyte molecules on an electrode start migrating all at once with a minimum dispersion. However, in the solution just above the electrode surface, the electric potential is uniform over the entire width of the electrode and there exists no electric field to drive the molecules in the direction tangential to the electrode surface, i.e., along the channel. Consequently, molecules that are initially in close proximity to the electrode surface leave gradually, which produces a wide dispersion of molecules as shown in Fig. 4(a). To minimize the dispersion, we should have applied a tricky voltage control, by which molecules are "launched" upward before the electrophoretic drive, as shown in Fig. 4(b). Thus, sound electric field that is free from the Debye screening and small dispersion of analyte molecules cannot be achieved simultaneously. These problems, though minor, may put limitations on the simplicity and efficiency of the present separation scheme.

3.2 Crosswise migration-type REM

To overcome the above-mentioned problems, the following modifications should be made.

(a) Electrophoretic migration of analyte molecules should be in the direction normal to the surface of the electrodes. This ensures that analyte molecules start their electrophoretic migration all at once, which makes the resolution of electrophoresis free from influences of the width of the electrodes, i.e., we do not need fine electrodes to obtain better resolution.

(b) Increase in area of electrodes makes the chip tough against the Debye screening because it requires more time and concentration of ions in the sample solution to form a shield of the electrode voltage.

The configuration of the chip and sorting schemes are modified in consideration of the above. Figure 5 shows the configuration of the crosswise migration type, which consists of double arrays of electrode at

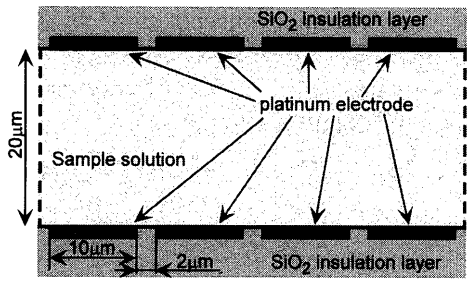


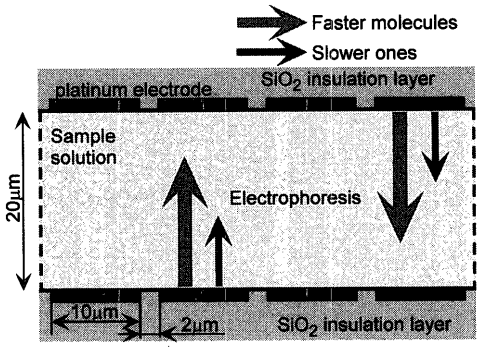
Fig. 5 Configuration of crosswise migration type

both the walls of the microchannel. The width of electrodes is larger with a smaller gap between them. The sorting scheme for this type of chip is shown in Fig. 6(a) - (d). Electrophoresis is carried out between a pair of electrodes that are facing across the channel, in the direction crossing the microchannel, while net motion of sorting transport occurs along the microchannel. Figure 6(a) shows the electrophoresis stage. Electrophoretic migration occurs in every two lanes between the facing electrodes in different directions. At a scheduled time, the analyte molecules are ratcheted (Fig. 6(b)). A peak of electric potential is generated in the middle of the electrophoresis line, which separates analyte molecules into those that have already passed the peak and those that have not. The former ones, those having electrophoretic mobilities larger than the threshold, are transported forward (upward in Fig. 6(b)) while the latter ones with smaller mobilities are transported backward (downward). After all of the analyte molecules are collected at the electrodes, they are transported to the next electrodes in the sorting transport stage (Fig. 6(c)). Molecules with larger mobilities are transported to the right, while those with smaller mobilities to the left. Now two sorts of molecules that come from different directions merge at an electrode and the next cycle begins right after this state (Fig. 6(d)).

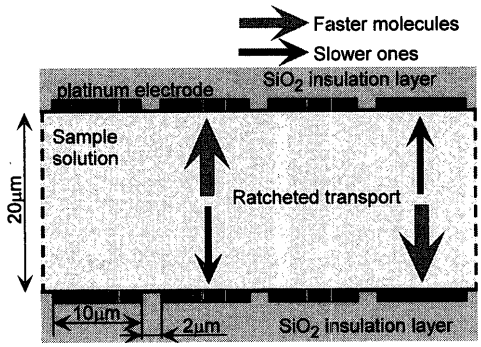
In such a way, a net motion to the right is produced for analyte molecules having mobilities larger than the threshold while the net motion is to the left for molecules with smaller mobilities. Thus, the chip is expected to have the same function as the single array system with better separation capability.

3.3 Separation and transport of DNA on the chip

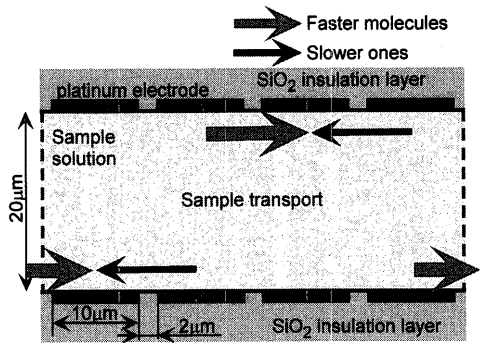
Transport and separation of DNA in the chip shown in Fig. 5 has been simulated using the system of Eqs. (1), (2) and (5). Two species of analyte molecules, DNA 45 mer and 55 mer, were assumed in the aqueous solution sample. The control scheme for the voltage of electrodes is shown in Table 2. Initially, just after the sample loading, the analyte molecules may be dispersed in the whole region of the



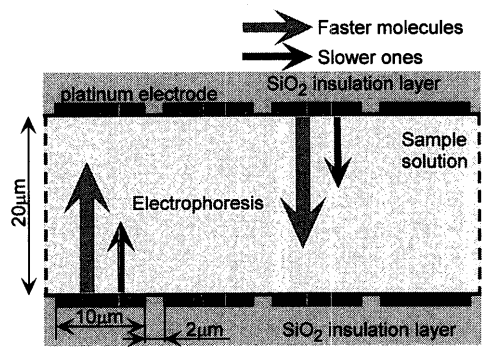
(a) Electrophoresis



(b) Ratcheted transport



(c) Sorting transport



(d) Electrophoresis in the next cycle

Fig. 6 Sorting scheme of the crosswise migration type

channel. Then, the analyte molecules are collected at two electrodes, 2 L and 4 U for example, and the ratcheting electrophoresis cycle begins. The molecular distribution approaches a steady cyclic state when the cycle is repeated, and then, a cycle with an identical molecular migration is repeated; the figure shows such a cycle. Molecules start their electrophoretic migration from the electrodes 2 L and 4 U. At the end of step 3-2, all the analyte molecules are collected at electrodes 1 L and 3 U. The next cycle starts from this state with the molecular location shifted to the left by one electrode as compared with the previous cycle. After four such cycles are completed, molecules are collected at 2 L and 4 U, which is identical to step 0 in Table 2.

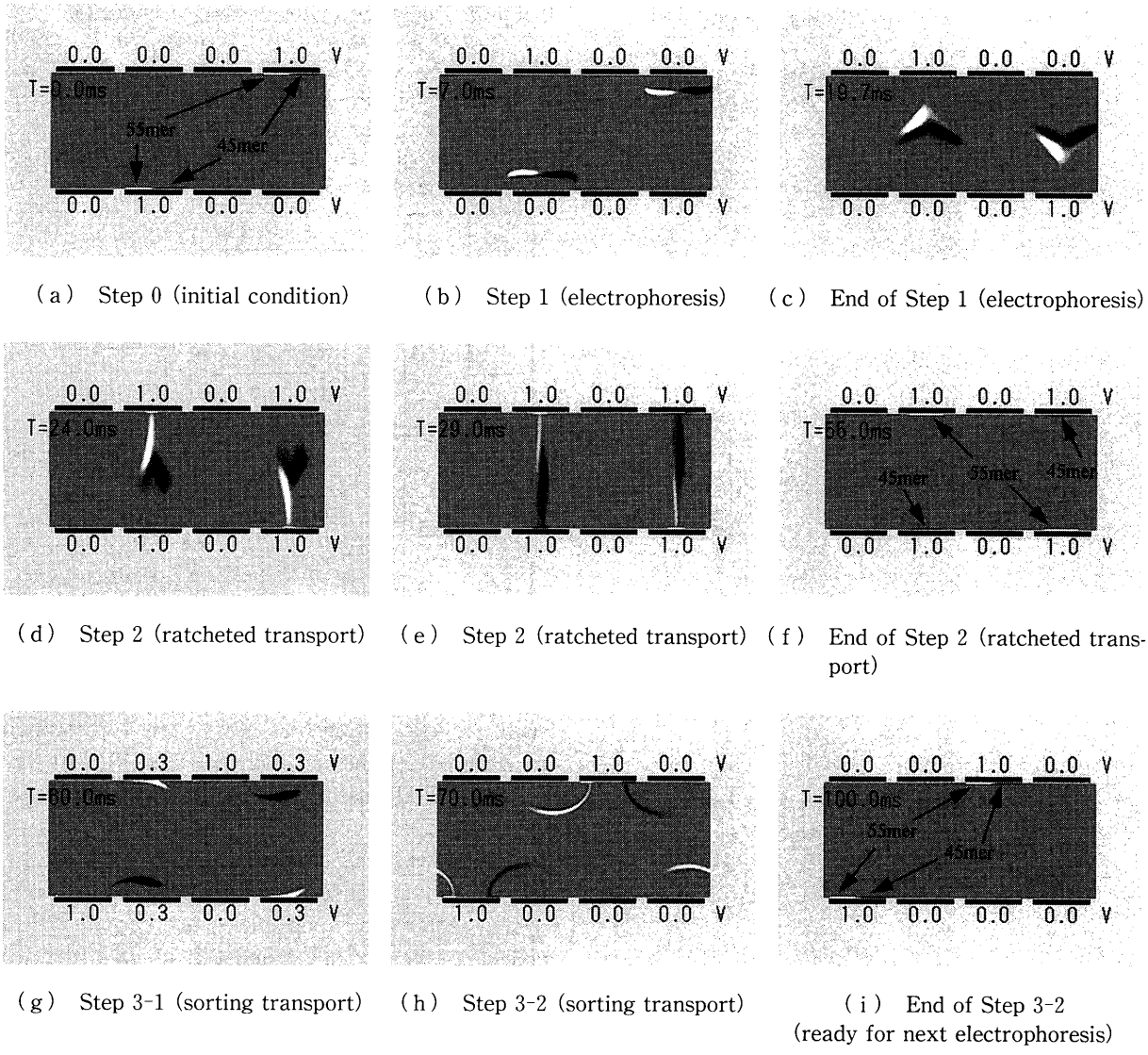
Table 2 Operation of the crosswise migration type for separation of DNA 45 mer and 55 mer. Electrodes on the upper wall of the channel in Figs. 5 and 6 are indicated as 1 U - 4 U from left to right, while those on the lower wall as 1 L - 4 L. Initially, all the analyte molecules are trapped at the electrodes 2 L and 4 U.

Step	Time (ms)	Voltage of electrodes (V) 1U / 1L	2U / 2L	3U / 3L	4U / 4L	Function
0	0	0 0	0 1.0	0 0	1.0 0	Initial condition
1	19.7	0 0	1.0 0	0 0	0 1.0	Electrophoresis (Fig. 6(a))
2	35.3	0 0	1.0 1.0	0 0	1.0 1.0	Ratcheted transport (Fig. 6(b))
3-1	12.5	0 1.0	0.3 0.3	1.0 0	0.3 0.3	Start of sorting transport (Fig. 6(c))
3-2	32.5	0 1.0	0 0	1.0 0	0 0	Sorting transport (Fig. 6(c))

The transport of the two species of DNA molecules is shown in Fig. 7. High-concentration areas of DNA 55 mer and 45 mer are indicated in white and black, respectively. It is clear when we compare Figs. 7(b) and (c) with Fig. 3 that electrophoresis with much less dispersion of analyte molecules is achieved. This improvement leads to better separation accuracy. The probability of analyte molecules being sorted to the correct position by a single electrophoresis cycle is now 75%, while it was 60% for the single array system[11].

4. Concluding Remarks

The basic mechanism of the ratcheting electrophoresis microchip (REM) proposed by the authors was presented and its performance was evaluated by a computational simulation considering transport of ions and transient electric potential field in aqueous solutions determined by the transient concentration distribution of ions. It was demonstrated that the REM has an excellent capability of separating biomolecules and it can be refined by the newly proposed crosswise migration type.



(a) Step 0 (initial condition) (b) Step 1 (electrophoresis) (c) End of Step 1 (electrophoresis)

(d) Step 2 (ratcheted transport) (e) Step 2 (ratcheted transport) (f) End of Step 2 (ratcheted transport)

(g) Step 3-1 (sorting transport) (h) Step 3-2 (sorting transport) (i) End of Step 3-2 (ready for next electrophoresis)

Fig. 7 Transport of DNA. Concentrations of DNA 55 mer and 45 mer are indicated in white and black, respectively.

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