Direct observation of λ-DNA molecule reversal movement within microfluidic channels under electric field with single molecule imaging technique*

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The electrodynamic characteristics of single DNA molecules moving within micro-/nano-fluidic channels are important in the design of biomedical chips and bimolecular sensors. In this study, the dynamic properties of λ-DNA molecules transferring along the microchannels driven by the external electrickinetic force were systemically investigated with the single molecule fluorescence imaging technique. The experimental results indicated that the velocity of DNA molecules was strictly dependent on the value of the applied electric field and the diameter of the channel. The larger the external electric field, the larger the velocity, and the more significant deformation of DNA molecules. More meaningfully, it was found that the moving directions of DNA molecules had two completely different directions: (i) along the direction of the external electric field, when the electric field intensity was smaller than a certain threshold value; (ii) opposite to the direction of the external electric field, when the electric field intensity was greater than the threshold electric field intensity. The reversal movement of DNA molecules was mainly determined by the competition between the electrophoresis force and the influence of electro-osmosis flow. These new findings will theoretically guide the practical application of fluidic channel sensors and lab-on-chips for precisely manipulating single DNA molecules.

Keywords: reversal movement, electrophoresis, electroosmosis, electric field threshold value

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1. Introduction

Over the last few decades, the rapid progresses of molecular biology, cell biology and single molecular imaging techniques have greatly promoted the developments of single-molecule biological science and technology.[1–5] Today, by combining the single-molecule biological science and the micro-/nano-scale confine techniques, researchers can directly investigate the statics dynamics and electrokinetical behaviors of a single bio-molecule in real-time.[6–9] Also with employing the micro-/nano-fluidic technologies, researchers have facilitated the separation, stretching, selection, mixing of DNA molecules, and can even visually study the interaction between DNA molecules in biomimetic environments.[10–14] In order to precisely manipulate a single DNA molecule, scientists have developed various kinds of functional structures and devices, such as T-junctions,[15] channel-arrays,[16] nano-scale slits,[17] and cross-slots.

Currently, micro-/nano-fluidic technologies have been widely employed to manipulate a single DNA molecule for understanding their various properties.[18–20] To drive DNA molecules within micro-/nano-fluidic channels, there are usually four kinds of mechanisms: (i) electro kinetic flow; (ii) pressure-driven flow; (iii) capillary effect; and (iv) diffusion.[21–23] Among these methods, both the capillary effect and diffusion are normally passive and hard to be controlled, and the pressure-driven flow has strict requirements for applied equipment.[22,24] The electrically driven flow is the most common and favorite method for researchers,[2,25] since it has many advantages such as being easy to control and fast to respond. However, there are still critical problems that have not been thoroughly comprehended. For example, how the velocity of DNA molecules changes when they move within the micro-/nano-fluidic channels. The complete investigations about these challenges will greatly advance the practical application of micro-/nano-scale fluidics and lab-on-chip techniques.

It is well known that the movement of a DNA molecule is quite complicated when it is driven through the micro-/nano-fluidic channels by an external electric field. The eventual moving velocity and direction are the comprehensive results of

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various effects, for example, electroosmosis, electrophoresis, and electroviscous.[24] In addition, the effect of the electrical double layer (EDL) of the wall and the DNA molecules cannot be ignored.[26] In fact, almost all of the relevant unprecedented phenomena are more or less related to the EDL effect when the bio-molecules move within the micro-/nano-fluidic channels.

Up to now, to measure the velocity distribution of the aqueous solution in micro-fluidic channels, effective techniques have been developed, such as micro particle imaging velocimetry (micro-PIV), particle tracking velocimetry (PTV), and molecular tagging velocimetry (MTV).[27–29] The basic principle of these methods is determined by calculating the velocity of tracer particles, which is considered to be equivalent to the velocity of fluid. In these techniques, polystyrene microspheres are widely used. However, the movements of DNA and other biological molecules in micro/nano-channels are very complicated. The results based on the aforementioned methods are not accurate enough to measure the velocity of DNA molecules. This is mainly because of the following essential differences: first, the DNA molecule is a soft material, its shape and configuration are not fixed but change with the properties of flow, the geometry of the channel, and the material of the channel wall; second, the interaction between the DNA molecules and the inner channel walls is also complex. In the micro-/nano-channel flow, the wall shear layer of flow can cause the intermittent tangle of DNA molecules. It is not conducive to continuously observing and investigating the DNA movements.[30–32] It has been found that the electrostatic force between the DNA molecule and the wall becomes more important and even plays the leading role when the ionic strength of the solution is low, regardless of the shape (e.g., square, rectangular, or triangular) of the micro-/nano-channel geometry.[33]

Combining the micro/nano-fluidics and the visualization techniques, scientists can characterize DNA molecules dynamically and non-destructively.[34–38] Bram *et al* have observed that a DNA molecule reeled in from both ends at the same time, and a kind of toroidal structure was likely to be formed under the tension. Xu *et al.*[39] found that when DNA molecules were compacted in the micro-channel fluidic device, DNA chains could be extended by flow forces; they also directly observed the deformation of a DNA chain by exposing it to the salt solution at the stagnation point.

In the 1990's, Chu's group systematically studied the relaxation of DNA molecules with the visualization techniques. They found that the DNA molecules could be stretched to full extension in a flow, and the visual length was exponentially decayed with time when the flow stopped.[40] They also studied the single polymer dynamics in an elongational flow and a steady shear flow, and found that the probability distribution of molecular extension was related with the strain rate, in contrast to the behavior in pure elongation flow, and the average polymer extension in shear flow did not display a sharp coil-stretch transition.[41,42]

Nowadays, benefited from the approaches of soft lithography, various functional microfluidic devices have been fabricated inexpensively and reliably with tailored geometries.[43–45] Among these micro-/nano-channels the most commonly used structure in laboratories is still the straight channel, which is also widely utilized in practical application.[46–49] In fact, when the straight micro-/nano-channels are employed to study the DNA conformations new phenomena are still discovered from time to time. Recently, Tang *et al.*[50] reported that the uniform direct current (DC) electric fields were able to compress the DNA molecules, and the DNA molecular conformation relied on the intensity of electric field and the ionic strength of solution rather than the DNA location in the channel.

In this investigation, by adopting the single molecule fluorescent inverted microscopy and EMCCD, we found a significant phenomenon in the micro-/nano-fluidic channel: under the external electric field the DNA molecule can move reversely. To the best of our knowledge, this phenomenon is firstly reported here.

2. Experiments

2.1. Experimental setup

The reversal characteristics of DNA molecules moving through micro-/nano-fluidic channels were studied by the inverted fluorescence microscopy. Figure 1 is a schematic diagram of the experimental setup. A 100-W mercury arc lamp (190–1100 nm) was used as the light source. The sample λ-DNA molecules were stained with fluorescent dye YOYO-1 which was excited by the light of 490 nm wavelength and emitted fluorescent light of 510 nm. The fluorescence was collected by the inverted fluorescence microscope (IX-70, Olympus Japan) with a series of objective lenses such as 20× (0.40 NA) and 60× (0.75 NA). The fluorescent signal was focused onto an electron multiplying charge coupled device (EMCCD, Evolve 512, ROPER, USA). The EMCCD can dramatically improve the signal intensity of the images (by amplifying the weak signal) with high speed. Finally, all the images were analyzed by the software Image J (http://rsbweb.nih.gov/ij/).

The processes of experiments can be described briefly as follows. Firstly, the micro-channel chip was fixed on the sample platform, and the anode was inserted into the *cis* chamber, while the cathode was inserted into the *trans* chamber. Then, the buffer solution was dropped into the *trans* chamber. In order to prevent the retraction of aqueous solution, a small amount of buffer solution was dropped into the *cis* chamber as soon as the channel was filled with the buffer solution.

After that, an appropriate amount of DNA solution was injected to both reservoirs. Subsequently, the power source was turned on, and an external DC voltage was applied to the two platinum electrodes, i.e., the electric field was established between the two poles. Finally, the movements of DNA-YOYO-1 molecules were analyzed in real-time.

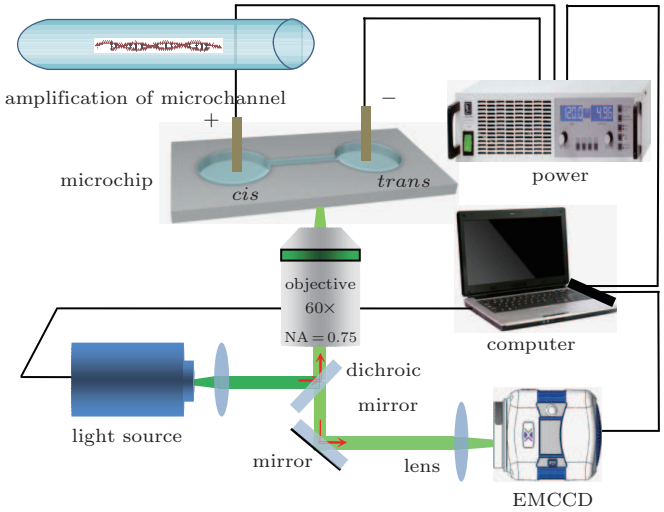


Fig. 1. (color online) Schematic diagram of experimental set-up.

2.2. Sample preparation

The λ-phage DNA molecules (48.502 kbp, Sino-American Biotechnology Company, China) were used in all experiments. They were dyed with fluorescent dye YOYO-1 (Molecular Probes Company, USA). The fluorescent dye was inserted into the λ-phage DNA molecule at the ratio of base pair: dye molecule = 10 : 1. DNA molecules were suspended in the buffer solution (10 mM Tris-HCL, PH = 8.0, 50 mM bis-tris, Calbiochem, Co., Germany). During experiments, 1000 μL Tris and 100 μL bis-tris were firstly put into two containers which were marked 1 and 2, respectively. Secondly, 0.1 μL YOYO-1 and 0.411 μL DNA original solutions were respectively poured into container 1 and container 2. After being well mixed, the two containers were placed into a dark chamber and educated about 30 min at room temperature. Thirdly, 200 μL solution was removed from container 1 and placed in container 2 and then mixed. In order to stain completely and uniformly, the mixture of YOYO-1 and DNA was further incubated for about 30 min at room temperature in the dark room. Finally the concentration of the DNA-YOYO-1 solution was about 0.455 mg/L.

For the sake of leading the DNA molecule solution into the micro-channels easily, the PLL(20)–g(3.6)–PEG(2) solution (ShangHai Yuan Ye Biological technology co., LTD, China) was used to treat the inner wall of the channel.[51] The PLL (20 kDa) and PEG (2 kDa) were mixed at the ratio of 1:3.6. The mixture of PLL–g–PEG was dissolved into the 10 mL MHEPES solution. The final concentration was 1 g/L, and the PH value was about 7.4.

2.3. Microfluidic chip

In the experiments, micro-fluidic channels with circular cross sections were used (Beijing Q&Q Technologies Co., Ltd). They are made by silica and have different inner diameters, which are 5 μm, 10 μm, and 300 μm, respectively. The lengths of all channels are about 5 mm.

Generally, the surface of the quartz tube is hydrophobic, and the silica substrates have the negatively charged surface at a PH value above 3 due to the deprotonation of the surface silanol (Si-OH) group. Because the PLL is positively charged, the PEG can be embedded into the backbone of PLL by electrostatic interaction. As a kind of neutral hydrophilic polymer, the PEG chain can effectively prevent the DNA molecules from nonspecifically being absorbed onto the inner wall of the channels.[51] Therefore, the PEG prevents the absorption of DNA molecules onto the channel inner surface.[52] This is very useful, since it is not only easy to guide the DNA molecules into channels, but also can reduce the influence of EDL.

The two reservoirs of samples were made with polymethyl methacrylate (PMMA) (chloroform: PMMA = 1 : 1). In order to strengthen the reservoirs and protect them from being damaged by the buffer solution, the upper surfaces of reservoirs were coated by a layer of glue (epoxy resin: polyamide resin = 1 : 1).

3. Results and discussion

The velocity of DNA molecules moving within microfluidic channels might be affected by multiple factors, such as the inner diameter of the channel, electric field intensity, PH value and the concentration of the buffer solution.[53] The different buffer will also influence the DNA molecules movement, so the body forces of different electrolytes in the microchannel were different.[54]

3.1. Reversal motion of DNA in the micro-fluidic channel with 10 μm diameter

The details of λ-DNA molecules transferring along the microfluidic channels with 10 μm diameter were systemically studied. During the experiments, the external applied voltages ranged from 0 V to 100 V. In other words, the electric field intensities were changed from 0 V/m to 1.33×10^4 V/m. When the applied electric field was smaller, about $E < 1.43 \times 10^3$ V/m, no DNA molecule was observed inside the channel. As the electric field intensity was gradually increased to about $E = 2.67 \times 10^3$ V/m, a few DNA molecules were observed moving through the channel. After that, with the electric field enhancing, the number of DNA molecules translocating through the channels increased.

Figure 2 shows a series of snapping images of DNA molecules moving from the *cis* chamber to the *trans* chamber under the applied electric field $E = 2.67 \times 10^3$ V/ m. It should be pointed out that the direction of DNA molecules

moving within the channels is in accordance with that of the applied electric field. This phenomenon is paradoxical. Because the DNA molecules are negatively charged, thus, the moving direction of DNA molecules driven by electrokinetic force should be in the opposite to the direction of the applied electric field. To the best of the authors' knowledge, this phenomenon is reported here for the first time.

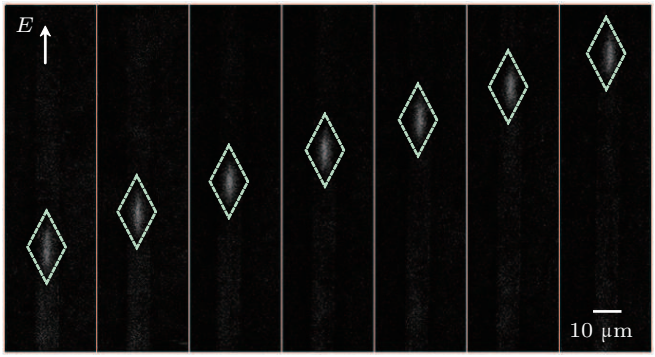


Fig. 2. (color online) The snapping images of DNA molecules moving from the *cis* chamber to the *trans* chamber along the microchannel with 10 μm diameter at $E = 2.67 \times 10^3$ V/m.

What causes this phenomenon? In general, when DNA molecules transfer within micro-/nano-fluidic channels under an external electric field, there are majorly four kinds of electrokinetic forces,[24] e.g., electroosmosis force (EOF), electrophoresis force (EP), dielectrophoresis and electrorotation. In addition, the diffusion effect may influence the DNA movement.

The dielectrophoresis is also called two-dimensional electrophoresis, this mechanical phenomenon is that one force is exerted on a low dielectric object when it is subjected to a non-uniform electric field.[55,56] The electrorotation is the circular movement of an electrically polarized particle;[57] it can be generated from a phase lag between an applied rotating electric field and the respective relaxation processes, e.g., when a small biological particle is put into the perpendicular electrode and the four electrodes provide AC signal. At the beginning of our experiment, benefiting from the capillary force, the microchannels were filled with buffer solutions. Therefore, except the electroosmosis and diffusion effect, other forces did not make a considerable contribution to guide the DNA molecules to transfer from the *cis* chamber to the *trans* chamber.

In order to judge what the dominant factor was for the reversal phenomenon, electroosmos or diffusion effect, other additional experiments were conducted at $E = 2.67 \times 10^3$ V/m. (i) The DNA solutions were only dropped into the *cis* chamber, then after about ten seconds, the DNA solutions were dropped into the *trans* chamber. It was found that the DNA molecules moved from the *cis* chamber to the *trans* chamber. (ii) The DNA solutions were dropped into both chambers, however, the order of adding samples was fastidious. That is, the DNA molecule solutions were firstly dropped into the *trans* chamber and then the *cis* chamber. The same results were found as in the case (i). DNA molecules moved from the *cis* chamber to the *trans* chamber. The reason for the DNA molecules translocating from the *cis* chamber to the *trans* chamber, for the experiment (i), may be attributed to the diffusion effect. However, the same result was observed from experiment (ii), where it was evidently beyond the diffusion effect. It is the electroosmosis force that can explicate the phenomenon reasonably, since the moving directions of negatively charged molecules driven by the electroosmotic force are the same direction with the applied electric field.

When the external electric field was continuously increased up to the $E = 4.0 \times 10^3$ V/m, the reversal phenomenon of the DNA molecule was observed as shown in Fig. 3. The movement of DNA molecule can be divided into three phases. First, when the DNA molecule just came into the view, defined the $t = 0$, in the time duration 0 s$< t <$ 6.282 s, the DNA molecules moved from the *cis* chamber to the *trans* chamber, the moving speed was continuously decreased. Second, when the time was about 6.282 s$< t <$ 9.423 s, the DNA molecules stopped moving and wriggled back and forth. Third, as the time $t >$ 9.423 s, the DNA molecules moved back from the *trans* chamber to the *cis* chamber, and the speed was gradually increased.

When the external electric field was further enhanced, i.e., the applied electric field was larger than 4.0×10^3 V/m, it was found that all the DNA molecules transferred from the *trans* chamber to the *cis* chamber. The moving direction of DNA molecule was opposite to the applied electric field.

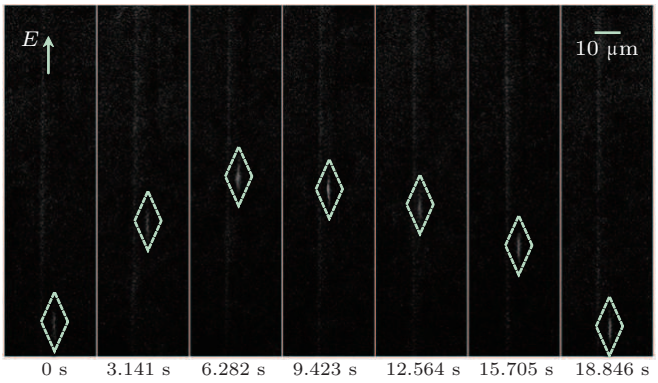


Fig. 3. (color online) The typical trajectory of reversion movement of DNA molecule in the microchannel of 10 μm diameter under applied electric field as $E = 4.0 \times 10^3$ V/m, the time interval between two adjacent pictures is approximately 3.141 s.

In general, when the DNA molecules are driven to move along the microchannels by the external applied electric field, the influences of electrophoresis and electroosmosis are existing simultaneously, however, they are unbalanced. Figure 4 schematically shows the competitive relationship between the electrophoresis force and electroosmotic flow effect that works on the DNA molecules.

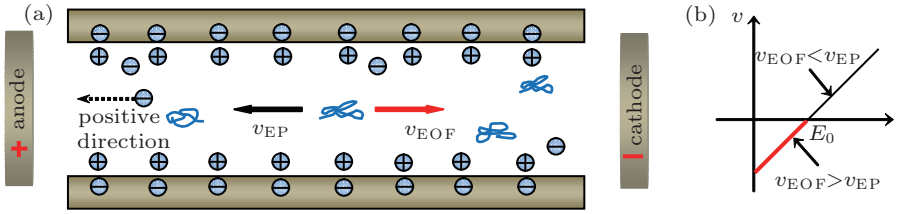


Fig. 4. (color online) Velocity direction of DNA molecule moving within the microchannel. (a) The schematic diagram of competition between electrophoresis and electroosmosis forces in the microchannel. The DNA molecule is negatively charged, the black arrow stands for the direction of electrophoresis velocity, while the red arrow is the velocity direction of electroosmotic flow. The movement direction of the negative ion under the electric field was defined as the positive direction of velocity (black dotted arrow). (b) When $v_{EOF} > v_{EP}$, the v_{real} of DNA molecules is negative. When $v_{EOF} < v_{EP}$, the v_{real} is a positive value.

When the motions of buffer solution and DNA molecules are both in a steady state, the overall velocity (v_{real}) of DNA molecule can be approximately obtained by summing up the velocity of electroosmotic flow (v_{EOF})[24,58] and the velocity of DNA due to electrophoresis force (v_{EP}),[24,59] and described as following:[27]

$$v_{EOF} = -\varepsilon_0 \varepsilon_r \zeta_{wall} E/\eta, \quad (1)$$

$$v_{EP} = \varepsilon_0 \varepsilon_r \zeta_{DNA} E/\eta, \quad (2)$$

$$v_{real} = v_{EP} + v_{EOF} = \varepsilon_0 \varepsilon_r E(\zeta_{DNA} - \zeta_{wall})/\eta, \quad (3)$$

where ε_0, ε_r, and η are the permittivity of free space, relative permittivity, and dynamic viscosity of solution, respectively, and ζ_{wall} and ζ_{DNA} are the zeta potential of wall and DNA molecules, respectively. Since a DNA molecule is a soft matter, it is flexible under external forcing, ζ_{DNA} may not be constant and should be different in different flows or external electric fields, etc. Therefore, the relationship between v_{real} and the external electric field intensity is complex, and this probably is the main reason why the moving direction of DNA was so different.

During the experiments, when $E < 4.0 \times 10^3$ V/m, the electroosmotic flow played a major role on driving the DNA molecules compared with the electrophoresis effect. The motion of DNA molecules was decided by the electroosmotic flow and the moving direction of the DNA molecule was consistent with that of the electric field. When $E > 4.0 \times 10^3$ V/m, the electrophoresis played a leading role, and then the motion states of DNA molecules were decided by the electrophoresis, the movement direction of the DNA molecules was the opposite direction to the electric field, due to the negative charges of DNA molecules. As the electric field intensity approaching 4.0×10^3 V/m, the electroosmotic effect became weaker than the effect of electrophoresis progressively and hence $v_{EOF} < v_{EP}$. The direction of the resultant force applied on the DNA molecules was opposite to that of the external electric field. Thus, the velocity of DNA molecules translocation along the electric field direction became slower and slower. After its speed value reached zero, then the DNA molecules moved along the opposite direction to that of the external electric field.

The value about 4.0×10^3 V/m was a special value that the translocation direction of DNA molecules was changed from the *cis* chamber to the *trans* chamber, just the reverse one compared to the initial direction. In order to facilitate the future research and analysis, the electric field $E_{tre,10} = 4.0 \times 10^3$ V/m is defined as the threshold value of the reversal movement of DNA molecules in the microchannel with 10 μm inner diameter.

3.2. Reversal motion of DNA in the micro-fluidic channel with 5 μm diameter

For the sake of verifying, the reversal motion of DNA molecule under an external electric field is a universal phenomenon in micro-fluidic channels, besides 10 μm diameter channel, other micro-/nano-channels with different diameters (300 μm and 5 μm) were also employed.

Figure 5 shows the motions of DNA molecules in three channels with different diameter: 300 μm (Fig. 5(a)), 10 μm (Fig. 5(b)), and 5 μm (Fig. 5(c)). It can be found from the Fig. 5(a), when the diameter of the channel was 300 μm, a large number of DNA molecules entered into the field of view so it was difficult to track an individual DNA molecule and was unreliable to calculate the corresponding velocity. However, when the diameter of channel was reduced to single micrometers, the individual DNA molecule could be easily captured as displayed in the Figs. 5(b) and 5(c).

Under the same working condition (i.e., the DNA molecules and the concentration of buffer solution), the smaller diameter of the channel, the larger the entropy of the DNA molecules needed to break through. Other research groups[60–62] have found the entropy is related with the diameter of channel, and the entropic trap decreased with the diameter of the channel. Han *et al.* studied the mobility of λ-phage DNA (48.5 kbp) moving within four different kinds of channels, and found that the smaller the channel diameter, the smaller of the DNA molecular mobility was, that is, the entropy was larger in the smaller diameter channel, so it would be more difficult for the DNA molecule to enter into the channel with smaller diameter.

Only when the entropy is large enough to exceed the threshold values, can the DNA molecule transfer into the fluidic-channel.

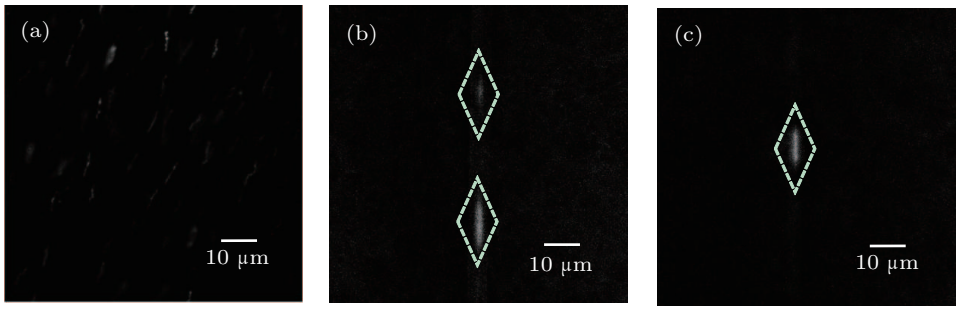


Fig. 5. (color online) The instantaneous movement images of DNA moving in the micro-fluidic channel with different diameters: (a) 300 μm, (b) 10 μm, and (c) 5 μm.

Figure 6 shows the detailed reversal movement of a single DNA molecule in the microchannel with 5 μm diameter. Figure 6(a) is a series of continuous images of DNA molecular movement around the reversion under the applied voltage of 40 V, i.e., $E_{\text{tre},5} = 5.3 \times 10^3$ V/m. Figure 6(b) schematically shows the reversal process of a DNA molecule corresponding to the Fig. 6(a). Similar to that in the 10 μm microchannel, the DNA reversion behavior in the 5 μm microchannel could also be divided into three stages. First, $0\ \text{s} < t < 41.88\ \text{s}$ the DNA molecule moved along the channel with the same direction as the electric field. Second, $41.88\ \text{s} < t < 62.88\ \text{s}$ the DNA molecule stagnated with oscillation back and forth. Third, $t > 62.88$ s, the DNA molecule moved along the channel in the opposite direction to the electric field.

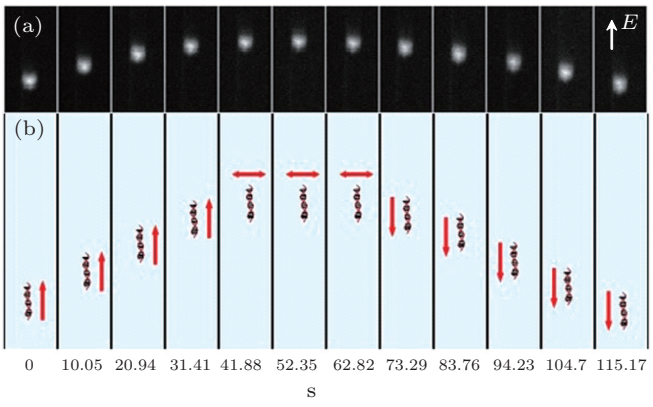


Fig. 6. (color online) Reversal removing of DNA molecule in 5 μm diameter microchannel under applied electric field 5.3×10^3 V/m. (a) Snap images, and (b) schematic diagram for analysis. The interval period between two adjacent pictures is 10.05 s. The red arrows stand for the moving direction of DNA molecule in the microchannel.

The reversal phenomenon of DNA molecules under applied electric field ($E = 5.3 \times 10^3$ V/m) as displayed in the Fig. 6, could also be explained by the competition between electrophoresis and electro-osmosis. When $E < 5.3 \times 10^3$ V/m, $v_{\text{EOF}} > v_{\text{EP}}$ electro-osmosis played a leading role. When $E > 5.3 \times 10^3$ V/m, $v_{\text{EOF}} < v_{\text{EP}}$ electrophores played a major part. Therefore, $E = 5.3 \times 10^3$ V/m was defined as the threshold value of electric field intensity for the DNA reversal movement in the channel with 5 μm inner diameter.

Comparing the reversal motions of DNA molecules in different inner diameter channels as described in Figs. 3 and 6, under the same sample concentration and PH value, the reversal threshold electric fields are different, 5.3×10^3 V/m and 4.0×10^3 V/m, corresponding to the microchannel diameter of 5 μm and 10 μm, respectively. It seems that the smaller the microchannel, the larger the threshold electric field is. However, no matter whether the 5 μm or 10 μm microchannel is used, the movement of the DNA molecules exhibit similar behaviors during the reversal period. Figure 7 displays the phenomena of inversion movement and the velocity of DNA molecules in the microchannels with different diameters: 5 μm (Fig. 7(a)) and 10 μm (Fig. 7(b)). It can be clearly found that in both microchannels, the velocities of DNA molecules are both negative firstly and then positive.

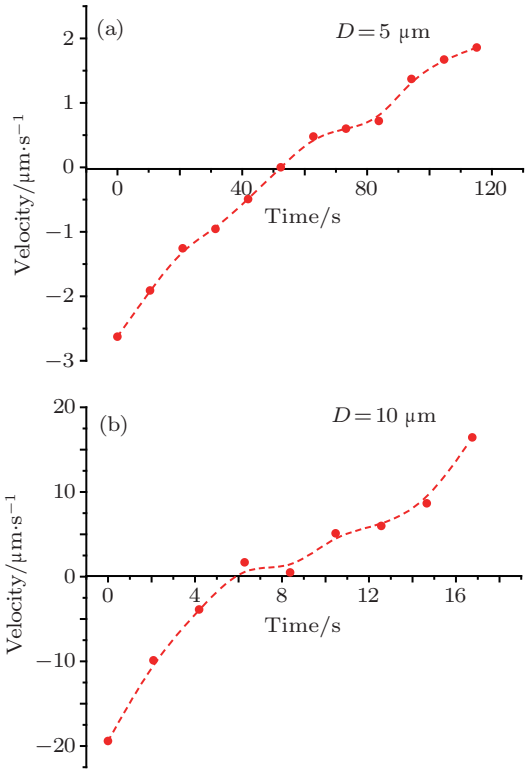


Fig. 7. (color online) Around the threshold electric field, $E = 5.3 \times 10^3$ V/m (5 μm) and $E = 4.0 \times 10^3$ V/m (10 μm), the velocity profile of DNA molecules reversal transferring in the channel with different inner diameter (a) 5 μm and (b) 10 μm.

To further demonstrate the relation between EP and EOF that cause the reversal behavior of DNA molecules in

microchannel, the accelerations of DNA molecules moving within microchannels were investigated as shown in Fig. 8. Figure 8(a) shows the relations of acceleration varying with time under three different electric field intensities, which are $E = 2.67 \times 10^3$ V/m, 4.0×10^3 V/m (the threshold value) and 5.3×10^3 V/m, respectively. When $E = 2.67 \times 10^3$ V/m, the electric field intensity is not large enough to generate the reversal motion of DNA molecule. Since the EOF under electric field is in the negative direction with stable velocity, the positive acceleration is directly applied on the DNA molecules due to EP. The EP force at this electric field is weak, the magnitude of acceleration is very small and close to zero (see Fig. 8(b) for reference). When the electric field is increased to $E = 4.0 \times 10^3$ V/m, the DNA molecule has deformation. The velocity of DNA molecule is significantly increased along the positive direction, and the acceleration is apparently larger than that of $E = 2.67 \times 10^3$ V/m. When $E = 5.3 \times 10^3$ V/m, the reversal position of DNA molecule moved towards the downstream of the field of view. What we obtained from the current field of view was the DNA molecule moving back in the positive direction. At this state, although the acceleration has similar magnitudes as in the case of $E = 2.67 \times 10^3$ V/m, the forces on the DNA molecule in the higher electric field case are different. In this case, as the moving direction of DNA molecule is different from that of EOF, except the EP force, the molecule is also dragged by the viscous force due to velocity gradient. Although the EP force is stronger due to higher electric field intensity, it is still approximately balanced by the viscous force. This is why there was no large acceleration as shown in Fig. 8(b). Although the reversal position is not captured in the field of view, when the electric field intensity is larger than the threshold value, the larger the electric field intensity, the further downstream the reversal position should be.

From Eqs. (1)–(3), it can be seen that the v_{EOF} is linearly determined by the electric field intensity, and the direction is nothing to do with the electric field intensity. The only reason that causes the reversal motion of DNA molecules is the changing of ζ_{DNA}, the electric field will induce the isotropic compression of the DNA molecules. The different electric field will produce different deformation of DNA molecules as being observed by Tang,[50] and the surface charge density of DNA molecules can be different. The ζ_{DNA} is influenced by the size and conformation of DNA molecule. The high electric field intensity seems to compress the DNA molecule and generate larger ζ_{DNA} than that of the DNA molecule in the natural case. This mechanism may support our experimental observations and explain the reversal movement of DNA molecule.

During the experiments, under the external electric field, the DNA molecules moving within the microchannel were not one uniform deformation. This phenomenon existed in both situations: under the same and different external electric fields.

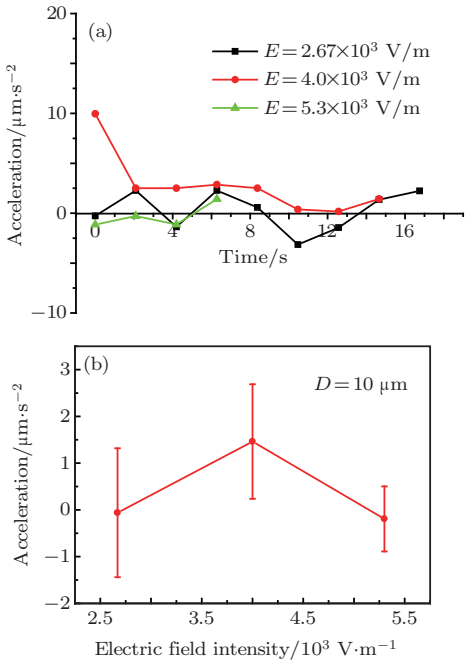


Fig. 8. (color online) The acceleration of DNA molecules translocating within 10 μm microchannel (a) the relationship of the acceleration versus time under different electric field intensities. (b) The relationship of the DNA molecular acceleration and the electric field intensity.

For the first situation, i.e., there were different deformations of DNA molecules under a different external electric field. Figure 9 shows the typical images of DNA molecules transferring within the microchannel with different deformation monitored by the EMCCD. Here, figures 9(a)–9(c) are the DNA molecules with different deformation, and the Figs. 9(d)–9(f) show the schematic diagram of the resultant force applied on the DNA molecules, corresponding to Figs. 9(a)–9(c), respectively. In Figs. 9(a)–9(c), all the marks (I) and (II) indicate two sequential images, the interval periods are all about 1.047 s. In Figs. 9(d)–9(f), the lengths of the arrows stand for the magnitudes of the forces.

It could be clearly found that during the same time, the distances of DNA molecules moving were different, $d_{\mathrm{a}}<d_{\mathrm{b}}<d_{\mathrm{c}}$, where d_{a}, d_{b}, d_{c} are the distances of the DNA molecules moving along microchannels for 1.047 s as shown in Figs. 9(a)–9(c), respectively. Thus the corresponding speed values were not uniform. With the change of the electric field intensity, the velocity gradient of fluid around DNA molecules was different. The different velocity gradient could induce the difference deformation of DNA molecules. In fact, the Weissenberg number is used to describe the DNA deformation in elongation: $Wi = \dot{\varepsilon}\tau$ here, $\dot{\varepsilon}$ is the velocity gradient of fluid along the direction of flow, τ is the characteristic parameter that represents a period of polymer transforming from Brownian motion to a state that completely rearranged its conformation, τ is usually a constant when the chemical and electrochemical characters of solution and the externally applied electric field intensity are unchanged. Therefore, the greater the $\dot{\varepsilon}$ is, the greater the DNA molecule's deformation is.

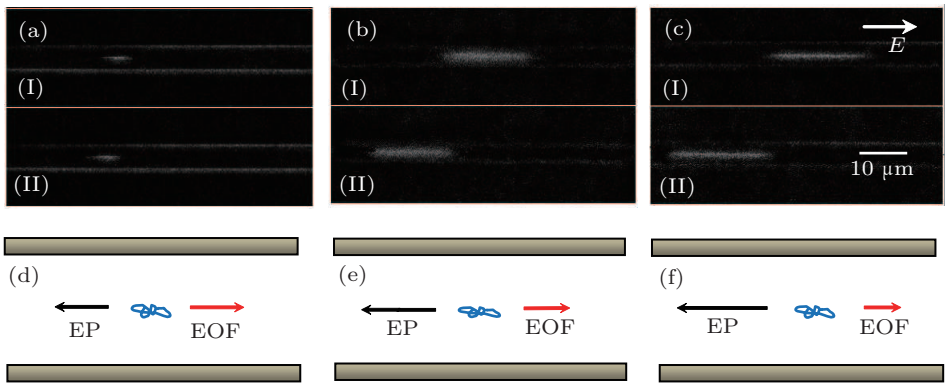


Fig. 9. (color online) DNA molecules translocate in the microchannel of 10 μm inner diameter with different electric field intensities. (a) $E = 4.0 \times 10^3$ V/m, (b) $E = 5.3 \times 10^3$ V/m, (c) $E = 6.67 \times 10^3$ V/m, the DNA molecules in panels (a), (b), and (c) move along the opposite direction to the electric field (the white arrow is the direction panels of electric field).

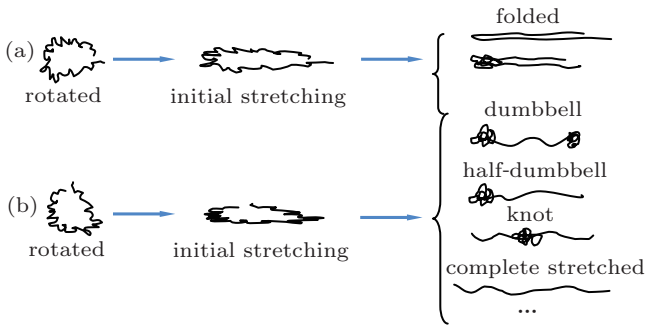


Fig. 10. (color online) The schematic diagram of deformation of DNA molecules translocating from the entrance into the inside of the microchannel. (a) When both ends of the DNA molecules are in the same side that is along the direction of fluid flow, they will be stretched by the viscosity of the fluid, (b) when the initial state of both ends of the DNA molecule is perpendicular to the direction of the flow, the conformation of DNA molecules enters into the channel.

For the second situation, the DNA molecules have different deformation under the same applied electric field intensity. When the DNA molecules are driven into the channel, the effect of the nozzle of microchannel and the internal electrostatic repulsion among DNA molecules would bring about the DNA molecule's conformation being rearranged, which leads to the different initial states of DNA molecules while entering into the channel. Figure 10 is a sketch map about the deformation of DNA molecules transferring from the entrance into the inside channel. It is well known that the DNA molecules are tangled before they enter into the micro-/nano-channel. When both ends of the DNA molecules are in the same side that is along the direction of fluid flow, they will be stretched by the viscosity of the fluid as shown in Fig. 10(a), however, the folded DNA molecules are often difficult to stretch.[2] When the initial state of both ends of the DNA molecule is perpendicular to the direction of the flow, the conformation of DNA molecules enters into the channel as shown in Fig. 10(b), the dumbbell, half-dumbbell and knot states would appear. Some of them may be stretched completely, which is the most expected phenomenon. In fact, the deformation of DNA molecules in the solution is very complex, and the DNA spatial structure is determined by multiple factors. The deformation of DNA will further affect the direction of DNA dipole, as well as its relationship with the external and internal electric field, which is similar to that demonstrated by the behavior of a water molecule in a carbon nanotube.[63] These influences will feedback to further affect the rotation, alignment and deformation of DNA molecule.

4. Conclusion

Based on the single molecule fluorescence imaging technique, the electrokinetic characteristics of λ-DNA molecules transferring within microfluidic channels were systemically studied. It was found that there existed a universal phenomenon of DNA molecule reversal motion. When the applied electric field intensity was smaller than the threshold value of reversal motion, the DNA molecules moved in the same direction as the external electric field. While the applied electric field was larger than the threshold value, the DNA molecules moved in the opposite direction to the external electric field. The reversal threshold values of 5 μm and 10 μm diameter channels are 5.3×10^3 V/m and 4.0×10^3 V/m, respectively. The direction of the DNA molecules movement is mainly determined by the competition between the electrophoresis force and the influence of electroosmotic flow. When the electric field is smaller, the electroosmotic flow is more important to drive the DNA molecules; while the electric field is larger, the electrophoresis force is the major role. In addition, it was found that the higher electric field compressed the DNA molecule and generated much larger ζ_{DNA} than that in the natural case, and the recorded deformation of DNA molecule is closely related to the velocity while moving in the microchannel. The larger the velocity is, the more significant the deformation of the DNA molecule is.

The reversal motion phenomenon and its threshold electric field intensity are important and helpful for the precise manipulation of a single DNA molecule within the micro-/nano-fluidic channel. These new findings will theoretically guide

the practical application of fluidic channel sensors and lab-on-chips based on the electrohydrodynamics and optofluidics.

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