Characterization and Optimization of an Entropic Trap for DNA Separation

Jongyoon Han and Harold G. Craighead

School of Applied and Engineering Physics, Cornell University, Ithaca, New York 14853

Recently, a microfabricated entropic trap array was demonstrated to be useful in separating large (5–200 kbp) DNA molecules efficiently (within ~30 min), by dc electrophoresis, on a microchip platform without a sieving matrix. This paper reports further development of the technique, with emphasis on optimizing separation selectivity and resolution. The interaction of DNA molecules with regularly spaced entropic barriers was modeled in order to predict the effect of changing various structural parameters. The selectivity (differential mobility) was shown to be dependent on the depth of deep and shallow channel regions, applied electric field, and number of entropic barriers. Experimental data were compared with the prediction of the model. It was expected from the model that, in the low-field (severe trapping) limit, separation resolution should depend only on the number of entropic traps. However, in reality, resolution did depend on the applied field because the relaxation of DNA is not achieved at high fields. The requirement and feasibility of megabase pair DNA separation with the entropic trap array device was discussed.

Direct current gel electrophoresis for double-stranded DNA separation in a chip environment has been commercialized recently, but the resolution suffers as the length of DNA increases. Separation of long (20 kbp–1 Mbp) double-stranded DNA molecules is still largely done by pulsed-field gel electrophoresis. It has been reported that capillary pulsed-field gel electrophoresis can achieve fast separation of double-stranded DNA even up to several megabase pairs. Also, separation of megabase pair DNA by dc gel electrophoresis was recently reported. However, the complexity related to the electrohydrodynamic instability of DNA under a high-frequency ac field has been a problem. It seems that the dynamics of DNA under an ac field is still not well understood, which limits the application of the technique. In addition, most previous efforts toward fast DNA separation used gel or polymer solution as a molecular sieve, which should be introduced into a capillary or a chip prior to the experiment and replaced after several runs. In addition to the problem of gel degradation during this gel-filling process, it is generally cumbersome to introduce (often viscous) gel into the channel of a highly integrated micro total analysis system (μTAS).

Recently, we introduced a new DNA separation system that utilizes the entropic trapping effect of long DNA molecules. The schematic diagram of the device, together with the graphical definition of various parameters, is shown in Figure 1. Many entropic barriers can be made in a microfluidic channel by alternating two different (deep and shallow) regions. DNA molecules migrating under an external driving force (electric field, for example) are retarded at the entropic barriers. The retardation or trapping time depends on the size of the molecule, which enables the size separation of DNA. It is interesting to note that longer DNA molecules are trapped for shorter time at a given condition. It was shown that the local deformation of DNA molecules at the interface between a deep and a shallow region is critical in the escape process. The activation energy of this deformation is independent of the length of the DNA molecule. However, larger DNA molecules have more monomers facing the shallow region and therefore have a higher probability to escape from the trap. Using the entropic trap array device, the authors have demonstrated a substantial increase in separation efficiency compared to other conventional techniques, while downsizing the system. Separation of DNA in the range of 1–200 kbp was achieved within typically 30 min, in a channel 1.5 cm long and 30 μm wide, using only a dc electric field, with no sieving gel in the channel. Further information on the device and the separation process can be found in the previous publications.

Perhaps the greatest advantage of a microfabricated analysis system is in the capability to modify or “fine-tune” the device for even higher efficiency. For the characterization and optimization of the device, it is essential to have a theoretical model for the separation process as well as experimental control over the sieving structure. The entropic trap system uses a regular, microfabricated...
structure as a molecular sieve, which makes theoretical modeling easy. Its structural parameters (which are comparable to the pore size of the gel) can be controlled easily and precisely. These advantages of the entropic trap array system would allow optimization of the device to various applications. In this report, we characterized various factors affecting the separation selectivity and the resolution of the entropic trap array device. We conducted a set of experiments with the devices that have different structural parameters, and the results were compared with the prediction from the theoretical model. The separation resolution of the device and its dependence on the electric field were investigated. Finally, design requirements for megabase pair DNA separation in the entropic trap array device were discussed.

EXPERIMENTAL SECTION

Fluorescence Measurement. Various DNA samples were purchased from Sigma (T2 and T7 DNA, St. Louis, MO), New England Biolab (M ono Cut M ix, Beverly, MA), and Gibco BRL (5-kb ladder, Rockville, M D). DNA molecules were reacted with YOYO-1 intercalating dye (M olecular Probes, Eugene, OR) for fluorescence observation. The dye-to-base pair ratio of the final working solution was ~1:10. Tris–borate–EDTA (TBE) buffer solution at 5× concentration was used as electrophoresis buffer. β-Mercaptoethanol was added to the working solution up to 4% (v/v) to prevent excessive photobleaching. Fluorescence measurement was done on an inverted microscope (Olympus IX-70, M elville, NY) equipped with an adequate fluorescence filter set (Omega Optical, Brattleboro, VT). A 40× microscope objective lens (NA = 0.5) was used for imaging DNA molecules in the device. The motion of DNA molecules was recorded by an ICCD camera (ICCD-350F from Videoscope International, Washington, DC) and then was analyzed later by a video image processor (DVP-32 from Instrutech Inc., Long Island, NY).

Device Fabrication and Electrophoresis. The entropic trap array device was fabricated on a Si wafer by standard photolithography processes, followed by anodic bonding of a Pyrex glass cover plate (1.0-mm thickness, E sco Products, Oak Ridge, N J). Detailed photolithography process information can be found elsewhere.11,12,14 After the bonding, several fluid reservoirs were attached at the openings of the chip. The entire channel was completely filled with buffer solution prior to the experiment. Bubble formation could be a problem at times, but we were able to fill the channel completely by inducing electroosmotic flow. The DNA sample loading, collection (focusing), and electrophoresis were done in the same way as described in the previous publication.11 Fluorescence intensity peaks were fitted to a Gaussian curve to obtain the position and the width of individual peaks. To characterize the impact of changing structural parameters on the entropic trap separation process, we made and tested several entropic trap array devices, differing only in a certain structural parameter.

Safety Considerations. Fabrication of microdevices requires extensive cleanroom processing, which includes chemical/plasma etching and general photolithography processes. All the necessary safety procedures should be followed in these processes. YOYO-1 dye could be mutagenic, and gloves should be worn while the solution is being manipulated. In applying high voltages for electrophoresis experiments, standard electrical safety precautions should be taken.

RESULT AND DISCUSSION

Theoretical Modeling. The authors have shown that the trapping lifetime of a DNA trapped in an entropic barrier can be written as the following Arrhenius-type equation.13

\[ \tau = \tau_0 \exp \left( \frac{\Delta F_{\text{max}}}{k_B T} \right) = \tau_0 \exp \left( \frac{\alpha}{E_{\text{av}} k_B T} \right) \]  

(1)

where \( \Delta F_{\text{max}} \) is the activation energy barrier for DNA escape. \( \Delta F_{\text{max}} \) indeed scales as \( \sim \alpha / E_s \) (where \( \alpha \) is a constant) and is not a function of the length (N) of DNA.13 The prefactor \( \tau_0 \), however, does depend on the length of DNA, which makes size-selective separation possible. The N dependence of \( \tau_0 \) is based on the fact that the escape (attempt) probability is proportional to the number of monomers facing the shallow region gap. Strictly speaking, one should know the conformation of DNA molecules trapped at an entropic barrier to calculate \( \tau_0 \) precisely. This is not easy, considering the field gradient at both ends of the deep region.

The conformation of a trapped DNA will be determined by the competition between the entropic elasticity of the molecule and the electrical driving force. In fact, \( \tau_0 \) is dependent on the electric field, since at a higher field DNA molecules are “pressed to the wall” in the entropic trap more severely. The field gradient would not only distort the equilibrium shape of the molecule but also render the distribution of monomers nonuniform over the molecule.

To make calculation easier, however, we first neglect the nonuniform monomer distribution due to the field gradient. Also, we assume a low-field regime, where the entropic elasticity mainly determines the conformation of the DNA. This approximation is corroborated by the fact that the electric field in the deep region (\( E_d \)) is negligible compared to that of the shallow region (\( E_s \)). The relations between \( E_s \), \( E_d \), and \( E_{\text{av}} \) are

\[ E_s = \frac{2d_s}{d_s + d_d} E_{\text{av}} = \frac{2\gamma}{1 + \gamma} E_{\text{av}} \]  

(2)

\[ E_d = \frac{2d_d}{d_s + d_d} E_{\text{av}} = \frac{2}{1 + \gamma} E_{\text{av}} \]  

(3)

Here, \( \gamma \) is the ratio between the deep and the shallow region depths (\( = d_d / d_s \)) and \( E_{\text{av}} \) is the average electric field applied to the device, which is distributed over the shallow and the deep

regions according to their relative thickness. In this experiment, \( \gamma \) was typically 20–50, which made \( E_d \) negligible compared to \( E_s \) or \( E_{av} \). The field quickly decreases as one moves away from the shallow region entrance. Since the size of the molecule is comparable to \( d_0 \), the majority of monomers of the trapped DNA feel only a negligible field. Then the shape of a trapped DNA can be approximated by a quarter-sphere, only bounded by the walls. The lateral width of this conformation (the radius of this quarter-sphere) should be proportional to \( R \) (the radius of gyration of a free DNA molecule).

One important characteristic of the electrophoresis of polymeric electrolytes is that molecules are "transparent" to the electric field. (The field line is not perturbed by the molecule.) Every monomer of a trapped DNA, lined up at the interface between the shallow and the deep region, should feel the same electric field and have the same probability of making the required transition for the escape. \( d_R \) is the area of the molecule facing the shallow region, and it is proportional to the number of monomers that attempt to make the transition. Therefore, the (escape) transition rate \( W \) of trapped molecules per unit time is \( W = d_R \exp(-\Delta F_{max}/k_B T) \). If one consider \( n \) trapped molecules, the change of \( n \) can be described by the equation of \( \text{dn} = -Wn \text{dt} \). The solution for this equation is an exponentially decreasing function of time, with the average lifetime of \( t \sim W^{-1} \). Therefore, the trapping lifetime of a DNA polymer trapped at a single entropic barrier is now given by the following equation.

\[
r \sim \frac{1}{d_R \exp\left(\frac{\alpha}{E_s k_B T}\right)} \sim \frac{1}{d_R n^{1/2} \exp\left(\frac{\alpha}{E_s k_B T}\right)}
\]

(Gaussian chain) (4)

One should note that eq 4 is only valid in the low-field regime (\( r \) should approach zero when the field is large). At high fields, \( \tau \) becomes comparable to or smaller than other relevant dynamical time scale (for example, relaxation time of a polymer). Equation 1 cannot be used to describe such a situation.15

There are some theoretical simplifications in the above model. Dielectrophoresis of DNA molecules should be considered where there is a field gradient such as the edge of the deep region. However, the net effect would be canceled out since at the other edge it will work in the opposite way. Also, when DNA molecules are stalled at the trap, there is local flow perturbation caused by the counterions.16 A trapped DNA will act as a small electroosmotic flow pump, causing a fluid flow around the molecule.17 This effect was also neglected due to the complicated nature of the problem.

The mobility \( \mu \) of a DNA molecule in the entropic trap array can be written as,

\[
\frac{\mu}{\mu_0} = \frac{t_{\text{travel}}}{t_{\text{travel}} + \tau} = \frac{1}{1 + \tau t_{\text{travel}}}
\]

where \( \mu_0 \) is the free draining mobility (the mobility of DNA when there is no entropic trapping effect). \( t_{\text{travel}} \) is the time for a DNA molecule to travel one shallow and one deep region, between trapping events. Here, DNA molecules are assumed to be "free-draining" in both deep and shallow regions, migrating with the same mobility \( \mu_0 \). Therefore, \( t_{\text{travel}} \) can be written,

\[
t_{\text{travel}} = \frac{L/2}{\mu_0 E_s} + \frac{L/2}{\mu_0 E_d} = \frac{L}{4\mu_0 E_{av}} \left( \gamma + 1 \right)^2
\]

(6)

From eqs 4–6, the mobility of DNA in an entropic trap array is given as,

\[
\frac{\mu}{\mu_0} = 1 + \tau t_{\text{travel}} = \frac{1}{1 + \beta \frac{4\mu_0 E_{av} \tau}{d_R L(\gamma + 1)^2} \exp(\alpha/E_s k_B T)}
\]

(7)

where \( \alpha \) and \( \beta \) are proportionality constants. Note that there is additional \( \gamma \) dependence in the exponential factor (through \( E_s \)). The term \( \tau t_{\text{travel}} \) is important. If \( \tau t_{\text{travel}} \ll 1 \), the trapping effect is insignificant and the size selectivity approaches zero. If \( \tau t_{\text{travel}} \gg 1 \), the mobility of DNA is low and it takes a long time for DNA bands to elute out of the channel.

Experimentally, the differential mobility of DNA (\( d\mu/dN \), selectivity) is more important that the mobility function \( \mu \) itself. In eq 7, the only \( N \) dependence comes from \( R \) term in the equation. Therefore,

\[
\frac{d\mu}{dN} = \frac{\mu_0}{N(1 + \tau t_{\text{travel}})^2}
\]

This means that the selectivity decreases as the length of the DNA increases.

**Characterization of the Entropic Trap Array Device.** Figure 2 is the graph that shows \( N \) dependence of the mobility in the entropic trap array device. The mobility was calculated from the DNA band elution time, and the solid line is the fitting to the model (eq 7) with the assumption of \( R \sim N^{1/2} \) (ideal chain model).

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The fitting starts to fail when the length of DNA becomes smaller than ~10 kbp. It is expected that our model would break down when the radius of gyration (R) approaches the shallow region thickness (d_s). Deviation of data points from the fitting shows that smaller DNA molecules are less severely trapped than expected from the model. Also, the slope of this graph decreases with N, which reflects ~1/N dependence in eq 8.

To verify the theoretical model, we did experiments where various experimental parameters were changed and results were compared with the model. E_av, d_d, and d_s were the parameters of interest in this study. To test d_d dependence, we prepared two similar devices that differed only in d_d and compared electropherograms of the same DNA sample. Peak positions are plotted against the elution time in Figure 3. Increasing d_d caused a decrease in the slope of the mobility versus N (DNA length) curve. Therefore, the size selectivity (d_d/u dN) of the entropic trap array device is a decreasing function of the d_d. This is in agreement with the theoretical model (eq 8). The d_d dependence of the r/t_travel is,

\[
\frac{r}{t_{\text{travel}}} \sim \frac{E_{\text{av}}}{d_d RL} \exp \left( \frac{\alpha}{E_{\text{av}} k_B T} \left( 1 + \frac{d_s}{d_d} \right) \right) \quad \text{(in the } \gamma \gg 1 \text{ limit)}
\]  

which is a decreasing function of d_d. Therefore, eq 8 also decreases with a increase in d_d when r/t_travel < 1. (In the experiments, r/t_travel was typically 0–0.5.)

The physical reason behind this trend is the following. If d_d is increased, this increases the aspect ratio (\gamma), which in turn increases E_d and decreases E_d. The increase in E_d will result in higher escape probability (contribution from the exponential factor of eq 9), since E_d is the driving force for the escape transition. At the same time, decrease in E_d will increase t_travel, which would make any trapping effect less significant (contribution from the prefactor of eq 9).

Changing d_d without changing other parameters has two conflicting effects on entropic trapping. Increase in d_d means that there are more monomers available at the shallow region gap, which would enhance the escape of DNA. On the other hand, the increase in d_d while keeping d_s constant will result in a decrease in \gamma, which will then decrease E_d and enhance entropic trapping. This can be seen from eq 8, where d_d dependence can be written as the following.

\[
\frac{r}{t_{\text{travel}}} \sim \frac{E_{\text{av}}}{d_d RL} \exp \left( \frac{\alpha}{E_{\text{av}} k_B T} \left( 1 + \frac{d_s}{d_d} \right) \right)
\]

The behavior of this function depends on the factor \Delta F_{\text{max}}/k_B T (= \alpha/E_{\text{av}} k_B T), which is the ratio between the energy barrier and the thermal energy. At typical conditions of this experiment, it is believed that the energy barrier \Delta F_{\text{max}} is comparable to the thermal energy k_B T (\Delta F_{\text{max}} \approx k_B T), since the electric field is usually set as high as possible for separation of a given species. (Such a low-trapping condition enhances the speed of the separation.) Therefore \Delta F_{\text{max}}/k_B T is rather close to, but larger than, 1. In that case, eq 10 decreases with increasing d_d (The function (1 + x)^{-2}e^{x(1 + x)} is a decreasing function near x = d_d/d_s = 0, \gamma \gg 1, when A < 2). Therefore, the increase of d_d will decrease the r/t_travel factor as well as the selectivity. This was also verified by the experimental data. The effect of changing d_d on separation selectivity is shown in Figure 4. When d_d of the device was increased from 75 to 100 nm, the separation selectivity decreased.

In Figures 3 and 4, the actual migration speed (or time) of DNA bands is less meaningful than the slope (the selectivity) of the curve. They are a comparison between data taken from two different devices. The actual speed of migration (or the free draining mobility) could be slightly affected by the chip-to-chip variation of the surface condition.

The period of the channel, L, as well as the ratio between the shallow and the deep region length, is another structural parameter that can be easily modified and characterized. The consideration about changing these two parameters is given below. (1) The period of the entropic trap array, L, is inversely proportional to the number of entropic traps per unit length of the channel. Therefore, with the same total length of the device, increased L would lead to a decrease in separation resolution, since DNA molecules would undergo fewer entropic traps. This can be clearly seen in Figure 5. In these electropherograms, DNA molecules up to 100 kbp were separated using entropic trap array devices with the same total length of the device, but separation resolution deteriorates with the increase in L. Note that the voltage of electrophoresis is lower than the previous runs with shorter DNA.
molecules. (2) The minimum \(L\) is limited by the fact that the deep regions should be at least as large as the DNA molecules to be separated. For the 40-kbp DNA molecules, channels with \(L = 2 \mu m\) showed poorer separation than channels with \(L = 4 \mu m\). It is believed that \(L\) should be larger than or comparable to the radius of gyration to ensure DNA is not compressed by the channel wall. This limitation becomes severer in separating larger DNA molecules. Also the minimum \(L\) is limited to \(\approx 2 \mu m\) due to the practical resolution limit of the photolithography process used in this experiment. (3) The period of the channel \(L\) was divided equally into a shallow and a deep region in this experiment, but this ratio can be also changed. In this experiment, only the equal division was used, but the effect of this change can be easily predicted by the following simple calculation. Let’s say the length of each deep and shallow regions are \(L_d\) and \(L(1 - \delta)\), respectively \((0 < \delta < 1; \text{see Figure 1})\). Then eqs 2, 3, and 6 are modified to,

\[
E_s = \gamma E_d = \frac{\gamma}{\delta + (1 - \delta)\gamma} E_{av} = \frac{E_{av}}{1 - \delta (\gamma - 1)/\gamma} \tag{11}
\]

\[
t_{travel} = \frac{L}{\mu_s E_{av}} \left(\frac{\gamma - 1)^2}{\gamma} - \delta^2 + \frac{\gamma}{\gamma - 1)^2} \right) \tag{12}
\]

\(E_s\) and \(E_d\) are the increasing functions of \(\delta\), while \(t_{travel}\) is maximized at the value of \(\delta = \sqrt{2}\) (the value used in this experiment). For better selectivity, one wants to decrease both \(t_{travel}\) and \(E_s\). Increasing \(\delta\) more than 0.5 will increase both \(E_s\) and \(E_d\), which will facilitate the DNA escape from the trap. It might be desirable to decrease \(\delta\) below 0.5 to get better selectivity, which would decrease both \(t_{travel}\) and \(E_s\). However, other constraints described above make this rather impractical. The length of a deep region should be large enough for sufficient relaxation of DNA molecule \((\approx 2 \mu m\) for 40-kbp DNA). One also needs to have as a small \(L\) as possible to get more entropic traps per given length, which limits the increase of the length of the shallow region. These variations can be studied in future experiments.

In Figure 5, DNA bands eluted out faster in a channel with shorter \(L\) value. This is probably either due to the lack of relaxation time in those channels or the effect of field gradient at the edge. Our current model does not account for the molecular relaxation process in detail, although it seems to play an important role.

**Dispersion and Separation Resolution of the Entropic Trap Array Device.** So far, we mainly investigated the selectivity of the entropic trap array device as a function of various parameters. However, separation resolution is a more important characteristic of a separation system. In the analysis of separation resolution, both the selectivity and the dispersion (band broadening) should be taken into consideration.

One important characteristic of the entropic trap array is that the diffusion of DNA is not a main source of dispersion. This is because entropic barriers virtually block DNA diffusion across the shallow regions. The spatial peak widths after separation runs turned out to be almost independent of the time DNA bands spend in the channel.\(^{14}\) It is believed that the dispersion of a peak in the entropic trap array device is largely caused by the statistical variation of the entropic trapping lifetime. The trapping time at an entropic trap follows an exponential distribution, and the probability of a DNA molecule to be trapped until time \(t\) is given as \(\approx \exp(-t/\tau)\). Then, both the average and the standard deviation of the trapping time would be \(\tau\).

If one considers two DNA peaks with the average trapping lifetimes of \(t_1\) and \(t_2\), respectively, the temporal difference of the two peaks is \(t_1 - t_2\) at the end of the channel, where \(n_0\) is the total number of the entropic traps in the device. At the same time, the standard deviation of the total traveling time for each peak would be \(n_0^{1/2}\tau_1\) and \(n_0^{1/2}\tau_2\), respectively. Therefore, the resolution of separation \(R_s\) at the end of the channel is given as,

\[
R_s = \frac{n_0(t_1 - t_2)}{\sqrt{n_0(t_1 + t_2)}} \approx \frac{\sqrt{n_0}}{2\tau} \frac{d\tau}{dN} \Delta N \tag{13}
\]

where \(\Delta N\) is the length difference between the two DNA species being separated. From the eq 4, one can obtain,

\[
R_s = \sqrt{\frac{n_0(\Delta N)}{2N}} \tag{14}
\]

Therefore, separation resolution can be enhanced simply by increasing the number of entropic traps of the device \(n_0\). Also, in the above equation, the resolution does not depend on the field
Figure 6. Separation of 5-kb ladder DNA at three different electric fields. L = 4 μm, d_s = 100 nm, and d_l = 1.8 μm.

(E) or any other structural parameters (d_l, d_s). Better selectivity of the entropic trap does not necessarily mean better separation resolution. Also, the resolution of separation scales with the inverse of N (just like the selectivity), which means that for longer DNA molecules, the separation resolution would be poorer. However, for a given fractional resolution (ΔN/N), the separation resolution is only dependent on the number of entropic traps.

Experimental data clearly shows that the entropic trapping separation is dependent on the electric field E. Figure 6 shows that separation resolution improves with a decrease in electric field. In separation runs with different electric field values in the same device, the lower voltage runs generally produce better-resolved electropherograms. This was a universal trend in all of the experiments with many different entropic trap array devices. Equation 14 is somewhat misleading since our theoretical model is based on the assumption that DNA molecules are fully relaxed before they are trapped. The relaxation time (t_relax) is known to be ~N^v, with v = 2 from the ideal chain model. For longer DNA, t_relax becomes comparable to or shorter than t_dyn (~1/E), which means DNA molecules are not fully relaxed. Also, in the high-field limit, DNA molecules can escape the trap before they are fully trapped (before all of their monomers arrive at the new trap) because the entropic barrier is almost negligible. In both cases, size selectivity would be lost and separation resolution would be poor. Therefore, for a given number of entropic traps and a certain length DNA, there exist a critical field value (E_c), above which the DNA molecule cannot be separated by the entropic trapping. This is corroborated by the observation in Figure 6 where the separation resolution of longer DNA bands (20–40 kbp) suffers with increasing electric field, while the separation of shorter DNA bands (5–20 kbp) is still maintained.

It is desirable to run at the lowest possible electric field for the maximum resolution possible. However, a lower electric field means lower DNA mobility, which increases the total electrophoresis run time. For example, in Figure 6, the electrophoresis at 47 V/cm took ~1 h while the run at 120 V/cm took less than 20 min. There is a tradeoff between resolution and speed of separation. Longer DNA molecules need to be separated at lower electric fields, while higher electric fields are acceptable for shorter DNA molecules. Therefore, the optimal electric field value should be selected according to the specific DNA length range. Assuming DNA relaxation as that of an ideal chain, the relaxation time of the DNA molecule would be ~N^2, while the trapping time would be given in eq 4. (In fact, the actual relaxation time of DNA in the channel would be shorter than that, since there is a field gradient in deep regions. After DNA passes a shallow region, the front end of the DNA moves slower than the back, leading to a field-assisted relaxation. The ideal chain model was used here to give an upper limit of the relaxation time of DNA.) By equating these two terms, one can get the relationship between the critical field E_c and the length of DNA.

$$\ln N \approx \frac{c_1}{E_c} - \frac{2}{5} \ln d_s + c_2 \quad (c_1, c_2: \text{constants}) \quad (15)$$

Effect of Electroosmotic Flow and Long-Term Stability.

The control of electroosmotic flow is essential in most microfluidic experiments. Figure 7 shows that the electroosmotic flow of the channel can be strong enough to disturb electrophoresis motion of DNA. At low buffer concentrations, DNA molecules moved toward the cathode even though they are negatively charged, due to strong electroosmotic flow. However, when the buffer concentration was increased, the effect of the electroosmotic flow diminished and DNA molecules moved toward the anode. At near 0.2 M buffer concentration (2X TBE), electrophoretic and electroosmotic driving force nearly canceled out, and DNA mobility became almost zero. However, at such a condition, a slight variation of surface charge in the channel could lead to an unstable

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Electroosmotic flow condition. As a result, a complex converging or diverging flow pattern as well as circulation could be observed. In this experiment, 5× concentration buffer was used to prevent this problem.

In many microfluidic separation and analysis systems, channel filling and surface treatment have been a major problem. Sometimes such preparation processes can be quite time-consuming even though the actual analysis is relatively fast. On the other hand, polymeric sieving materials usually degrade after a number of analysis runs, requiring a renewal of the sieving material or limiting it as a single-use device. Long-term stability and reusability of the entropic trap array device are important in practical application of the technique. Figure 8 demonstrates the long-term stability of the device. Here, two electropherograms from the same device, taken at times 6 months apart, are shown. During the period, the device was refilled with deionized water, stored in a refrigerator for an extended time, and reused with different samples many times. Still the device can generate virtually the same separation result, with only a small shift in overall mobility (i.e., a small shift in μav). The shift in μav was probably caused by the slight difference in the actual buffer concentration in two experiments (possibly due to evaporation). In addition to the same separation capability, the device did not show any sign of serious clogging or degradation. It was always possible to flush the remaining DNA molecule from the channel by applying higher fields. This result suggests that our system can be prepared as a "prefilled" device, which could be stored for a long time before it is reused.

**Toward Megabase Pair DNA Separation.** To enhance separation efficiency further and get better resolution, one can always increase the number of entropic traps (or make the device longer) for DNA molecules of any size. However, this is not always desirable considering the integration of separation channel with other components in TAS. Then, the question becomes whether it is possible to get better resolution by only changing the device structure. Equation 14 suggests that, as long as the entropic trapping time is significantly longer than the DNA relaxation time, resolution does not depend on electric field or device structure. Changing d4 or d5 might bring better selectivity in separation, but the peaks will be as much broader and separation resolution would be about the same. According to this, the only way to improve separation resolution would be increasing n1 (and the length of the channel).

Even though there is no field dependence in eq 14, the electric field Eav is a critical parameter for the separation of DNA molecules. The maximum field Ei at which one can get length-dependent selectivity depends on the length of DNA. Therefore, for shorter DNA molecules (5–20 kbp), Eav could be possibly increased further to enhance the speed of separation without affecting the separation resolution. In our experiment, Eav can be increased only up to 130 V/cm, because of the electrical breakdown problem between the Si substrate and buffer solution through the oxide. Electrical breakdown of the oxide also limits the maximum length of the device or, equivalently, the number of entropic traps (n1). Devices made on insulating substrates (glass or plastic) could be used to avert this problem. Also, longer channels could be fabricated into a small area by making multiple U-turns.

Application of entropic trap separation of midsize DNA fragments (50–20 kbp) would be feasible, even though capillary or chip-based gel separation is already available as an alternative technique. The main advantage of the entropic trap system is that it does not require any sieving matrix to be loaded and replaced. The sieving structure (entropic barrier) is very robust and stable over a long period of time. Speed of separation is also comparable to the capillary separation and could be improved by increasing the electric field. For smaller DNA molecules, however, thinner shallow regions might be necessary to achieve efficient trapping.

Another important question is whether the entropic trap array device can be used to separate very long DNA molecules, from megabase pair range to chromosomal DNA. Relaxation time is longer for larger DNA molecules, which means one should use much lower fields to ensure that entropic trapping is effective. On the basis of the theoretical model and experimental data, one can estimate the Ei and other critical parameters required for the separation of megabasepair DNA.

For the three different electrophoresis runs in Figure 6, there exists a critical length N where separation resolution becomes less than a certain value (~1). Roughly they are 17.5, 22.5, and 37.5 kbp for 100, 60 and 46.7 V/cm runs, respectively. Using eq 15, one can extrapolate the critical field Ei for longer DNA molecules. For a 2-M bp DNA (~50 times longer than iDNA), Ei would be ~20 V/cm. Considering the separation result of up to 100-kbp DNA molecules using the same device at 40 V/cm (Figure 5), this estimate seems reasonable. With an increase in DNA length, one has to consider the scaling of the entropic trap structure as well. Since the entropic trap should be large enough to contain a relaxed DNA molecule in the deep region, its dimensional scaling should be as ~N1/2. The diameter (size) of a 2-M bp DNA molecule is expected to be ~7 μm, assuming 1–2 μm diameter for the 40-kbp DNA molecule. To accommodate these large DNA molecules, the deep regions should be lengthened ~7 times (L should be increased ~3.5 times. The shallow region length does not have to be increased). Therefore, to keep the number of entropic traps the same as the current device (1.5 cm/4 μm ≈ 3700) to achieve required resolution (~1), the overall length

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![Figure 8. Long-term stability of the entropic trap array device. In both cases, Eav = 80 V/cm, d1 = 75 nm, and d2 = 1.8 μm.](image)

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of the device should be \( \sim 5 \) cm. In terms of time scaling, the band elution time scales as \( \sim L/E_c \). The first peaks in the electrophoresis run eluted out in \( \sim 10 \) min at \( 120 \) V/cm. At \( E_c = 20 \) V/cm, the elution of the least-trapped first DNA peak, in a 5-cm-long device, will take \( \sim 3.5 \) h. This is still better than the conventional pulsed field gel electrophoresis, which can take up to 24–48 h.

For the megabase pair DNA regime, we believe that the entropic trap array device can contribute to DNA purification, which may be more important than analytical separation. Here, the problem of peak dispersion can be solved simply by isolating the desired peak and repeating the process multiple times. The entropic trap device can be an efficient way to extract long DNA molecules out of a mixture of broken or damaged fragments. And in this application, efficiency can be improved just by optimizing the structure for better selectivity, which was extensively studied in this work.

Presumably, entropic trap separation could be further improved by a pulsed or ac field electrophoresis method. In addition, more complex field patterns can be applied to the entropic trap array to augment separation resolution, just as various pulsed-field electrophoresis techniques. (As an example of using pulsed field with entropic traps, see Slater et al.\(^\text{20}\)) DNA manipulation and separation using entropic recoil effect has been recently demonstrated,\(^\text{21}\) and this scheme could also be applied to the entropic trapping array device.

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