Invited Paper

From microfluidics to nanofluidics : DNA separation using nanofluidic entropic trap array device

Jongyoon Han and Harold G. Craighead*

School of Applied and Engineering Physics, Cornell University, Ithaca, NY 14853

ABSTRACT

Fluidic devices with sub-micrometer dimensions provide new opportunities in manipulation and analysis of various biomolecules, such as deoxyribonucleic acid (DNA). As an example of such devices, a microchannel with an array of 'entropic traps' is introduced. The existence of sub-100nm constriction causes long double-stranded DNA molecules to be entropically trapped, and the length-dependent escape of DNA from the trap enables a band separation of DNA. Entropic traps are also used to manipulate and collect many DNA molecules into a narrow, well-defined initial band for electrophoresis launching. In addition to its speed and compactness, another important advantage of this artificial separation device over conventional gel electrophoresis is the ability to modify and control the device precisely for the optimization of a separation process. The similar device could be used to analyze proteins or other biopolymers.

Keywords : separation, DNA, nanofluidics, entropic trap, biopolymer, electrophoresis

1. INTRODUCTION

Molecular separation is one of the most crucial techniques available in biology and associated technology. Biological systems usually consist of many levels of substructure, such as cells, nuclei and organelles. These subsystems are composed of many different biomolecules, such as proteins and DNA. The interaction between these biomolecules determines all the complicated biological phenomena of the system. In order to understand the interaction of these components, one should first isolate the components under study from the milieu of other biomolecules, eliminating the complexities from the environment. In many cases the very beginning of a biology experiment is separation (purification) of the target molecules from organisms.[1]

Cell components	Dimension
Human red blood cell	8 μm
Human nerve cell process (diameter)	1 μm
Mitochondria	~500 nm
Lysosome	200~500 nm
Secretory vesicles	50~200 nm
Virus particles	50 ~ 100 nm
Nuclear pore	~ 30 nm
Ribosome	~20 nm
Microtubules (diameter)	25 nm
DNA wrapped with histones	11 nm
Immunoglobulin G	5 ~ 10 nm

Table 1 : Dimension of various cell components (adapted from ref. (1))

The concept of a micro Total Analysis System[2] (μ-TAS, also called as a 'lab-on-a-chip') opened a new possibility in analytical sciences. The rapid advance of microfabrication technology for the microelectronics industry enabled one to efficiently make small objects on a wafer surface, which in turn was used to scale down analytical apparatus to tens of micrometers. Scaling laws work favorably in many cases, yielding greatly improved efficiency and accuracy.[2] In addition, miniaturized systems could analyze a small number of molecules, which is important in biological analysis where target molecules are sometimes scientifically or economically hard to produce in abundance. Interest in this field has been growing exponentially for the last several years, in variety as well as in quantity and quality.[3]

* Correspondence: Email: hgc1@cornell.edu; WWW: http://www.hgc.cornell.edu; Telephone: 607 255 8707; Fax 607 255 7658

Most microfluidic systems demonstrated so far have dimensions much larger then 1 micrometer, which is still much larger than the dimension of various biologically interesting particles or organelles, not to mention proteins. Table 1 lists some important cell components and their lateral dimensions. One can expect that a fluidic system with dimensions comparable to these cell components (<100 nm) would behave in a quite different manner than its larger counterparts. In other words, in such a small system, the interaction between the fluidic structure (or inner surface of the fluidic device) and the analyte in the fluid plays a major role instead of the interaction between the analyte and surrounding fluid. This brings us additional controllability in nanofluidics, because the fluidic structure is relatively easy to control and manipulate, while the fluid-analyte interaction is not.

Electrophoresis and choromatography are the most frequently used techniques to separate various types of biomolecules, although for some types of molecules these techniques cannot provide enough selectivity. Use of a sieving matrix such as gel is a way to solve this problem. Even though a porous sieving matrix can provide good resolution in separation analysis, the microscopic structure of these systems is inherently random, and critical dimensional parameters (pore size) are not easy to measure or control. This poses a problem in theoretical and experimental studies, aimed at improving and optimizing the technique for better separation.

Double-stranded DNA separation by gel electrophoresis is a good example of this situation. Separation of long double-stranded DNA (1kbp ~ 1Mbp) is important in many applications such as DNA fingerprinting. In conventional gel electrophoresis, DNA molecules are separated during an electric-field-driven motion in a gel because their mobility is dependent on their length. However, this length dependence of DNA mobility vanishes for long DNA molecules, mainly because the molecules tend to be more stretched and oriented in the direction of the electric field.[4] There have been many research efforts aimed to solve this problems,[5] including microfabricated systems.[6-8] From the previous work, it has become clear that a good understanding of sieving structure and its interaction with DNA molecules is required to achieve efficient separation of DNA. Gel-free nanofluidic electrophoresis channels with submicrometer-size constrictions can solve this problem.[9,10]

2. ENTROPIC TRAPPING OF DNA AT NANOFLUIDIC CONSTRICTION

Fig. 1 presents the idea of entropic trap arrays. The basic idea is to fabricate a device with constrictions narrow enough to 'filter out' DNA molecules. In free solution, DNA molecules assume a spherical shape described by the radius of gyration. The radius of gyration of lambda DNA (48.5kbp) is about 1 μm. Therefore constrictions much smaller than this size will be required to sieve DNA effectively. In addition to the constriction gap size (t_s), the depth of thick regions (t_d) is another

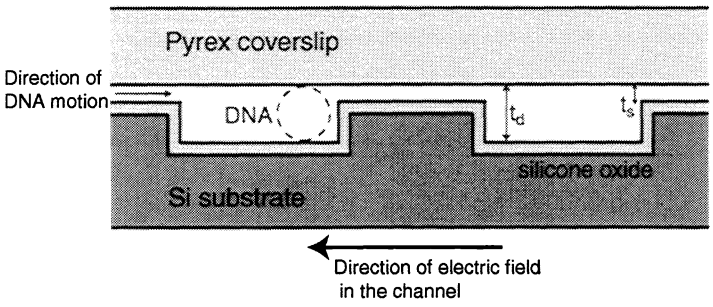

Fig. 1 : Entropic trap device for DNA. The radius of gyration of typical DNA is comparable to the depth of thick regions (t_d : 1 ~ 5 μm) , while the gap size of constriction (t_s : 50 ~ 100 nm) is considerably smaller. DNA molecules should be substantially deformed to enter the constriction, which requires a driving force provided by an electric field. The thick and thin regions are alternately repeated along the channel, and the length of each thin and thick regions are equal.

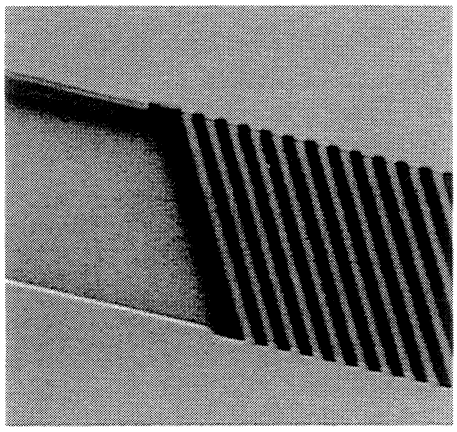

Fig. 2 : SEM of nanofluidic channel. It is the beginning of alternating thick and thin regions. The channel is 30 μm wide, and each thick and thin region is 2 μm long. (Period : 4 μm)

important parameter in device operation. For efficient separation and sieving, it is essential to make sure that DNA molecules return to their relaxed spherical shape. In pulsed field gel electrophoresis, this is achieved by switching off the field and waiting, which makes this technique quite slow. In entropic trap device, the thick region depth is larger than the radius of gyration of the DNA molecule to be separated, which makes the DNA relax faster.

We fabricated the constrictions in the vertical (z) direction using multi-level photolithography followed by differential etching of the thick and thin regions.[9] After etching the thin and thick regions to different depths, the Si wafer was thermally oxidized to provide electrical insulation between solution and the substrate. Finally, anodic bonding was used to bond a top plate (a glass coverslip) to form an enclosed channel. This fabrication method is easier than fabricating lateral constrictions with e-beam lithography. Also it is straightforward to fabricate the entropic traps over a large area, which is desirable for high-resolution separation. The finished device was filled with standard electrophoresis buffer (Tris-Borate-EDTA, TBE), and the motion of DNA was observed by fluorescence microscopy through the transparent cover plate.

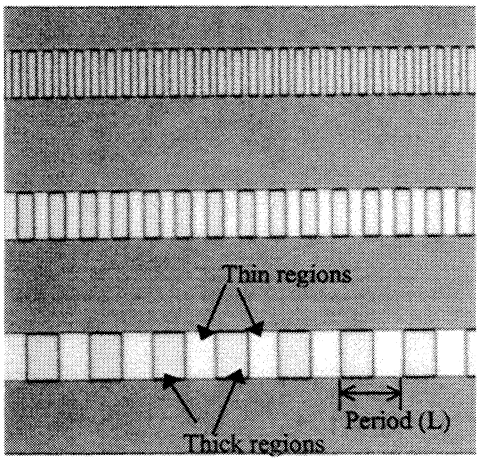

Fig. 3 Optical micrograph of finished device. Thin and thick regions are denoted. Three channels with different structural periods (L=8μm, 16μm and 32μm from the top) are shown.

Previously, the authors published a paper[10] about the dynamics of a single DNA moving through the entropic traps by electrophoresis. It was shown that DNA molecules are trapped whenever they meet the edge of the thin region, and the trapping lifetime is a strong function of the driving electric field. It is interesting that larger molecules can escape entropic traps faster than smaller molecules in this device. This is somewhat counter-intuitive considering the mobility in gel electrophoresis, where shorter molecule's mobility is higher. Obviously the separation mechanism here is different from that of gel electrophoresis, and the simplicity of the device permits one to construct simple physical model to explain this phenomenon.[10] The escape of DNA from an entropic trap is initiated by a local deformation of a trapped DNA molecule, which is unrelated to the rest of the molecule. The larger molecule has larger contact area with the thin region, therefore has higher escape attempt probability. This 'inverted' order of separation might have other interesting applications, such as purifying long DNA molecules from sheared or damaged fragments.

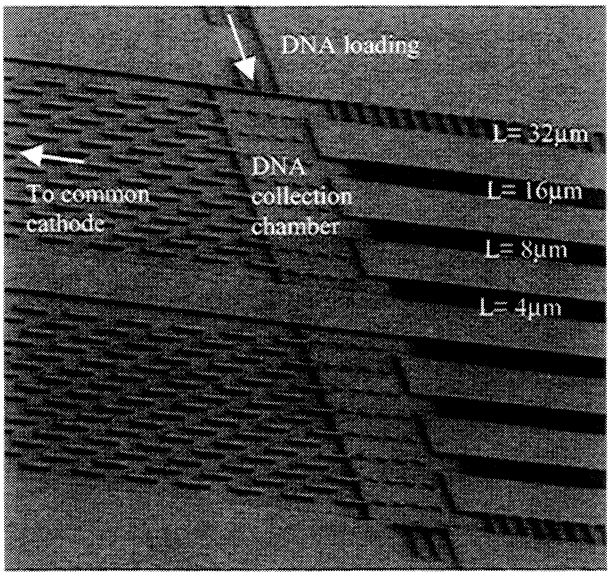

Fig. 4 : The loading region of two-lane entropic trap separation device. There are two separate but identical sets of 4 channels, side by side, for simultaneous analysis. Small channels on the right side of this SEM image are channels with different periods (L=32, 16, 8, 4 μm, from the outside). They are connected to a common DNA collection chamber. The central collection chamber is bounded by an entropic barrier from the loading channel and the cathode channel. The existence of this entropic barrier helps to manipulate and collect DNA molecules in the collection chamber. Other small posts in the central chamber and lines in the cathode channel are for preventing collapse of the glass cover plate during the anodic bonding. The channels with many thin regions are 30 μm wide, which is narrow enough to prevent any collapsing in the thin region during the bonding.

4. DNA SEPARATION USING ENTROPIC TRAP ARRAY DEVICE

In electrophoresis, it is desirable to have highly concentrated and focused initial band (plug). The entropic trapping mechanism could also be used to collect and focus lots of DNA molecules into a narrow band. When the applied electric field is low enough, DNA molecules cannot overcome even a single entropic barrier. One can trap many DNA molecules in this way, yielding a high concentration initial band to be launched. We fabricated a three-terminal DNA loading and collection system at the each end of the channel, which is shown in Fig. 4. Two different samples can be loaded into two separate sets of channels, allowing simultaneous analysis and comparison of electrophoregrams. In a recent publication,[9] the authors used this device to separate standard DNA ladder samples, and reported approximately 60-fold increase in speed compared with conventional pulsed field gel electrophoresis.

One of the most important advantages of the entropic trap device over gel is that it is possible to modify the structure of entropic trap precisely in order to get better separation. For example, the thin gap thickness (t_s, corresponding to the pore size of gel) can be varied precisely, while in gel electrophoresis the pore size can be controlled only indirectly by changing the concentration of gel solution. We designed a set of experiments to get information about the relation between several structural parameters (t_s, t_d and period L) and the separation efficiency. Some data from this optimization experiment are presented here.

For the characterization of different entropic trap devices, we fabricated several different devices with different t_s and t_d values. Also in the single device one can get data from four different channels with different L value (see Fig. 4). Driving electric field is also an important parameter to be varied for optimization. We used standard DNA ladder samples, purchased

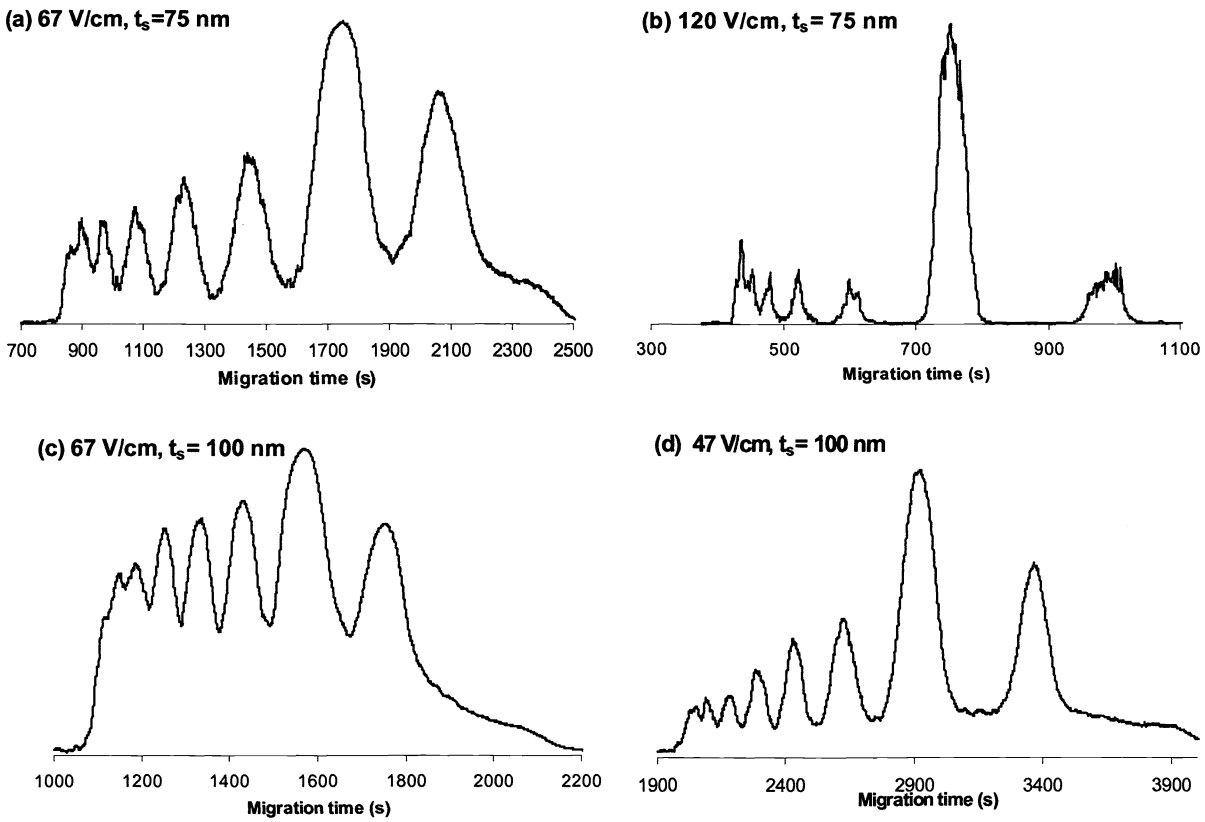

Fig. 5 : Electrophoregrams of a 5kb ladder separated in entropic trap array device. This sample contains double stranded DNA of 5, 10, 15, 20, 25, 30, 35 and 40 kbp. The 10kbp band is a reference band, showing a higher-intensity peak than the others. In all four electrophoregrams, the thickness of the thick regions was the same. (t_d = 1.8μm) (a) and (b) are the electrophoregrams from a device with t_s= 75 nm, and (c) and (d) are results from a device with t_s = 100nm. The difference in baseline between (a) and (b) is due to the different gain settings of the ICCD camera, and does not have any physical meaning.

from New England Biolabs and Gibco BRL. They were fluorescence-labeled with YOYO-1 (from Molecular Probes) at a dye to base pair ratio of 1:10. DNA solutions were then loaded into small reservoirs connected to the channel. After DNA molecules are loaded into the collection chamber of the loading region (Fig. 4), they were collected at the very first entropic barrier by applying small electric field between the anode and cathode. By this method, narrow bands of DNA were collected, typically less than ~50 μm wide. For launching, electric field value was suddenly increased to a working value, allowing collected DNA band to escape the first barrier and migrate through the whole channel. At the end of the channel, arrival of DNA bands were monitored with fluorescence microscopy, and the fluorescence signal from the channel was recorded by an intensified charge coupled device camera (ICCD, ICCD-350F from Videoscope International). A more detailed explanation of the experimental conditions is available elsewhere.[9,11]

From the previous single molecule experiment,[10,11] it was expected that the operation of entropic trap device is strongly dependent on the electric field. At high electric field, DNA molecules are not separated well because they do not spend enough time in the thick regions, where DNA molecules are relaxed after they go through the thin regions. We checked this by separating a 5kb ladder sample (5 ~ 40 kbp, from Gibco BRL) in the entropic trap device. In Fig. 5, (a) and (b) are electrophoregrams taken in the same device (t_s= 75 nm, t_d= 1.8 μm) at two different electric field values. One can see that the separation resolution worsens with the increasing electric field value. However, this deterioration of separation is also dependent on the molecule size to be separated, since the relaxation time of a polymer is proportional to the cube of its length.[12] Therefore the separation of shorter fragments is preserved while longer molecules are not separated in high electric fields. (Fig. 5-(b)) Higher electric field leads to faster separation speed, though. A tradeoff between separation resolution and speed must be considered in actual separation process, and the electric field should be adjusted according to the length range of DNA to be separated.

Comparison between Fig. 5-(a) and (c) demonstrates the effect of changing thin region thickness (t_s). At the same electric field value, the device with thinner thin region (t_s= 75 nm) had a slightly better resolution than the device with t_s= 100 nm. This was observed in electrophoresis runs obtained at several different electric field values. Earlier data with single molecule mobility showed that the length dependent mobility was completely lost when t_s ~500 nm. In our lab, we have fabricated and successfully filled the channel with t_s values as small as 50 nm. It is expected that current fabrication technique will allow for devices with even smaller t_s values. Even with relatively large thin region gaps, one could get better separation by further decreasing the driving electric field. Fig. 5-(d) is the electrophoregram obtained with the device of t_s=100 nm at 47 V/cm, showing improved peak resolution. However this was possible at the cost of separation speed, and it took about an hour for the last DNA band to complete the migration through the 1.5-cm long channel. Still, it is impressive considering that similar separation with pulsed field gel electrophoresis would take typically more than 10 hours.

From the above result, one can expect that, at any given electric field value, separation resolution worsens as the length of the molecule increases. Again, this can be explained by the length dependence of the DNA relaxation time. There should be a critical DNA length above which all the DNA molecules migrate together without being separated. To investigate the separation of longer DNA molecules, we used another DNA ladder with longer DNA strands (Midrange II markers from New England Biolabs). The electrophoregram is shown in Fig. 6-(a), at 40 V/cm with a device of t_s =100 nm and t_d = 1.8 μm. (Note that a lower electric field is required to resolve longer DNA peaks.) However the decrease in speed by decreasing electric field is not severe, mainly because longer DNA has higher mobility and can easily escape from the trap. In this electrophoregram, peaks of DNA longer than ~100kbp merged into a single peak and were not resolved. Further decrease in electric field or use of a longer channel with more entropic traps could resolve these peaks.

Fig. 6-(b) and (c) are the electrophoregrams obtained simultaneously with (a), but from channels with different structural period (L). Increased L means smaller number of total entropic traps for DNA to be trapped. Therefore, electrophoregrams (b) and (c) show poorer resolution than (a). It is also expected that the increased L will also increase the time allowed for DNA molecules to relax in the thick regions, which might be desirable for separation. In this particular situation (Fig. 6), the effect of decreased number of entropic traps is more significant than the improved relaxation in channels with larger L. However, this will depends on the electric field, t_d and other conditions, and probably more experiments are required to fully characterize these factors.

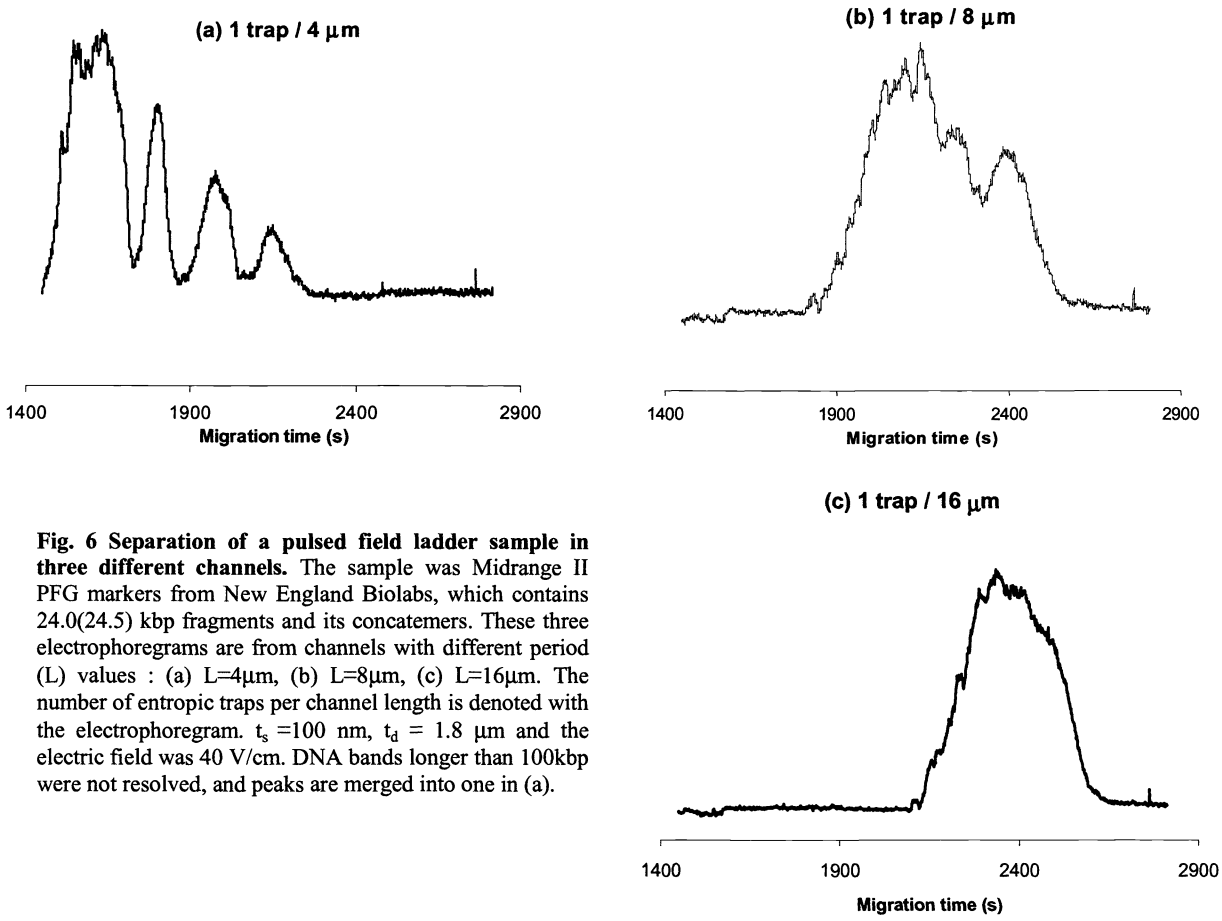

Fig. 6 Separation of a pulsed field ladder sample in three different channels. The sample was Midrange II PFG markers from New England Biolabs, which contains 24.0(24.5) kbp fragments and its concatemers. These three electrophoregrams are from channels with different period (L) values : (a) L=4μm, (b) L=8μm, (c) L=16μm. The number of entropic traps per channel length is denoted with the electrophoregram. t_s =100 nm, t_d = 1.8 μm and the electric field was 40 V/cm. DNA bands longer than 100kbp were not resolved, and peaks are merged into one in (a).

5. CONCLUSION

Nanofluidic device with sub-micrometer size constriction was used to separate double stranded DNA molecules according to their sizes. Although the free-flow electrophoresis mobility for DNA is length-independent, adding constrictions, similar-size to the molecular dimension, allowed efficient separation of DNA. This is possible due to the interaction between the artificially made, adjustable sieving structure and the DNA molecules, instead of fluid-molecule interaction as in the conventional electrophoresis. Further improvement can be achieved by adjusting several experimental parameters, which are directly connected with different aspect of separation mechanism. The ability to modify and adjust the sieving structure is essential in various application of this technique, such as DNA and protein separation at different length scales. Considering that most of important cell components are of sub-micrometer size, this could be applied to integrated cell analysis system capable of lysing a cell and sorting various cell components.

The fact that this system does not require gel or other separation matrix, as well as the compactness of the system, will work favorably in designing an integrated total analysis system. In fact, the real potential of this system will be fully exercised when the separation system is combined with sample preparation system. Separating extremely long DNA (~ 1Mbp) or chromosomal DNA is of great interest, and one of the most serious problems is the handing of very long DNA without shearing it. Microchannel environments offer ideal environment for this because of their turbulence-free nature. Ideally, chromosomal DNA could be extracted within a channel from the cell, transported to a reaction chamber, and then brought to the separation channel where the different reaction fragments are analyzed. Various nanofluidic systems could be integral parts in such a system, working as a separation or sample preparation schemes.

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