Physics of Semiconductor Devices

IWPSD-2003

Proceedings of the
Twelfth International Workshop on the
Physics of Semiconductor Devices

Volume 1

Editors
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Proceedings of the Twelfth International Workshop on the Physics of Semiconductor Devices: IWPSD 2003, Dec.16-20, 2003, Indian Institute of Technology, Madras, India

Nanofluidic Devices for Genomic Analysis

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We describe the use of micro- and nano-fluidic structures for the manipulation, sizing, and probing of biological molecules. The analysis time required for conventional genomic analysis approaches, such as pulsed-field electrophoresis, can be reduced by orders of magnitude. The structures enable new physical capabilities such as the continuous fractionation of DNA and the direct scanning of single molecules.

I. Introduction

Micro- and nano-fabricated structures offer many possibilities to improve our ability to manipulate and probe biological molecules such as DNA and proteins [1]-[7]. A first advantage of such approaches over conventional methods is that they can greatly increase the speed and integration levels and reduce the cost of conventional methods, enabling more widespread applications such as clinical point-of-care analysis. Second, the ability to manipulate and analyze single molecules enables one to study single molecules and the contents of single cells, enabling one to probe the heterogeneity that inherently exists within even genetically identical populations. Third, the small physical size can be used to enable methods not possible with conventional analysis, such as direct spatial imaging within genomes.

In microfluidic approaches for manipulating biological molecules, the conventional test tubes and pipettes are replaced by miniaturized plumbing channels (with 0.1- to 100-micron dimensions) etched into the surface of a wafer using methods borrowed from the integrated circuit industry, such as photolithography and reactive ion etching [1], [3]-[7]. In the simplest approaches, the tops of channels are sealed by bonding a flat cover slip to the top of the etched structure. Not only does this approach reduce the analysis volumes, but the integration of electrodes also enables one to use electric fields to drive ion and molecular flows (termed "electrophoresis") in addition to the possible application of pressure.

In this paper, we review recent results for both sizing [13], [16] and for linear scanning of DNA molecules [20]. Sorting based on size is a frequent step in sequencing, and more generally can be used to isolate desired genomic segments for further analysis. Because typical times for such operations are on the order of hours, they stand to greatly benefit from microfabricated approaches. A platform technology for integrated microfluidic analysis chips is presented, including its use in a "DNA Prism" for the 15-second sizing of large DNA fragments [13]. We also describe experiments using nanostructures for the direct physical inspection and scanning of single DNA molecules [19]-[20]. In these structures, nano-channels are used to force DNA into a linear state so that it can be directly imaged by either near-field or far-field methods.

II. Platform Technology for Integrated Microfluidic Sorting

The standard method for fractionating DNA fragments larger than ~40 kb, pulsed-field gel electrophoresis (PFGE), is extremely time-consuming, with running times of typically more than 10 hr [8], [9]. Several physical concepts have been developed for replacing PFGE with a microfluidic approach based on a microfabricated central array [10]-[13]. However, their practical implementation has been limited by the lack of a platform which can provide uniform electric fields and/or flows in tunable directions over the central array and also provide for the integrated loading of the source as well as extraction of the products.

Conventionally, two pairs of electrodes are used to create tunable fields in a two-dimensional area (Fig. 1a), one pair for each field component (vertical or horizontal). However, one pair of electrodes perturbs the field generated by the other pair, because electrodes impose equi-potential boundary conditions. This problem can be solved by using many electrodes clamped at voltages that approximate the voltage boundary conditions [9]. However, this method is impractical in microfluidic devices because many electrodes have to be used, leading to a packaging problem. Further, electrodes usually interfere with other functions of a device, such as sample loading and extraction, and they generate bubbles that bring the microfluidic device to a halt.

This limitation can be overcome by replacing the voltage-source (Dirichlet) boundary condition with a current-source (Neumann) boundary condition, and is implemented by surrounding the central array with hundreds of microfluidic channels instead of electrodes (Fig. 1b) [10]. These channels are electrical resistors due to the conductivity of the ionic buffers used as carriers of the DNA. When driven by a common voltage source, they act as electrical current sources when their resistances are made high compared to that of the central array. Therefore, correct boundary conditions for uniform vertical or horizontal fields can be imposed. Fields at an arbitrary orientation

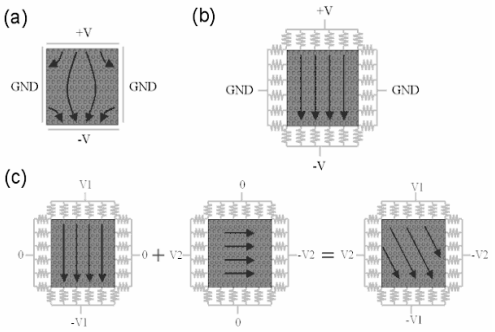

Fig 1. (a) Problem of field shunting when electrodes are fabricated on all sides of a central array, leading to non-uniform fields. (b) Current-injection boundary condition to achieve uniform fields, when peripheral resistors are large compared to that of the central array. The large resistor values insure negligible current (and thus field) flowing in/out of the sides of the device and thus a purely vertical field. (c) Generation of field at arbitrary angle by superposition of conditions for vertical and horizontal fields. [10]

can be created using superposition of conditions for horizontal and vertical fields (Fig. 1c). This method eliminates the need for electrodes inside the microfluidic device—they can be applied at the fluid reservoirs. Moreover, only one external electrode per side is needed in principle, although multiple electrodes lower the voltage requirement.

To load a narrow band of DNA into such structures for analysis, an extra channel must be added on one side of the array. Electrical fields (or hydrostatic pressure) can then be used to load the source material for analysis from a separate external reservoir.

III. DNA "PRISM"

The platform technology was then applied along with a central array of microposts in a quartz substrate to create a chip for the continuous rapid fractionation of DNA on the scale of 50 to 200 kb [13]. The central array consists of hexagonally-packed micron-scale posts (Fig. 2). It can be operated in two modes: conventional pulsed-field batch mode [11], [12] and the continuous-flow (DNA "prism") mode [13], which we describe here. As DNA molecules move through the posts, driven by the electric field, the pulsed electric fields in different directions (~10 Hz) force the DNA to uncoil and stretch out. A key physical concept is that the DNA gets pulled back and forth in the channels created by the post arrays, and when the fields are oriented at >90 degrees, longer molecules must travel further than shorter at each field shift before they can travel in the new direction. In this way shorter molecules move faster in the gel [11].

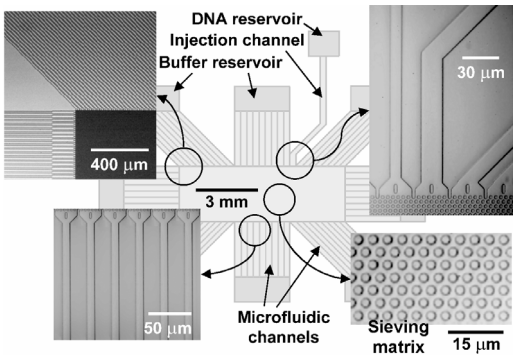

Fig. 2. Structure of the DNA Prism illustrating the microfabricated sieving matrix integrated with the microfluidic channels. The post array is 3 mm x 9 mm, and the posts are 2 μm in diameter, 2 μm apart, and 2 μm tall. A single channel connecting to the DNA reservoir injects DNA through a 28 μm opening. The many microfluidic channels around the array periphery act as resistors for realizing tunable uniform fields with a minimum of external contacts. [10], [13]

Most significantly, if the two electric pulses are applied at different strengths, the ability of smaller molecules to reverse direction can be exploited to cause molecules of different size to also move in different directions (Fig. 3a). Therefore, in the DNA Prism [13], DNA is continuously injected at the top of the array, and then flows down through it in a direction based on size (Fig. 3b). Flowing vertically over a distance of ~1 mm, DNA in the range of 50 to 200 kp has been separated in times on the order of 15 sec. This compares to some 10 hr for conventional PFGE, representing a speed-up of over three orders of magnitude [13].

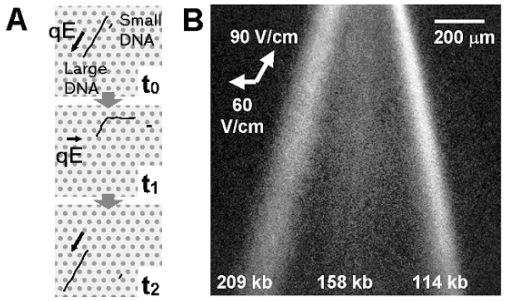

Fig. 3. (a) Schematic showing the behavior of small and large DNA molecules in post arrays through a full cycle asymmetric alternating-angle electric fields. Note that because the large DNA backtracks on a post when the field switches, the net direction of large and small DNA is different. (b) Operation of DNA Prism to show separation of 3 species of DNA (114, 158, and 209 kb) injected at a common point just above the field of view of the image. The fluorescent image (15 s after initial injection) shows the motion of the three DNA bands that result. Field strength oscillates between 90 and 60 V/cm in the direction shown at 3 Hz. [13]

IV. Brownian Ratchet Arrays

Unlike DNA molecules, many biological molecules, including proteins, are not linear polymers, and thus cannot be separated by the DNA prism. Brownian ratchet arrays do not require stretching of the molecules—globular molecules and molecules of other shapes can be sorted in the same run according only to their diffusion coefficients [14]-[16]. The Brownian ratchet array works by biasing diffusion; Brownian motion to one side is blocked by microfabricated obstacles, whereas diffusion to the other side is allowed and amplified. In practice, the ratcheting effect is achieved using obstacles tilted at an angle with respect to the flow (Fig. 4a), and samples are continuously fractionated as they flow through the course of obstacles.

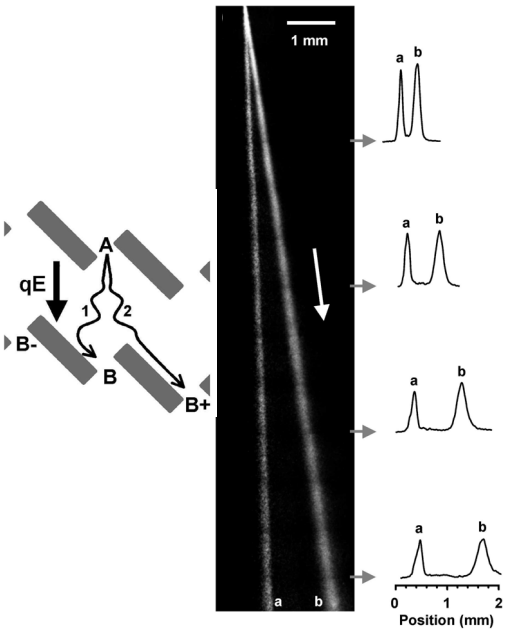

Fig. 4. (a) Basic principle of the Brownian ratchet array. As particles (molecules) flow down through the array of tilted obstacles (driven by electrophoretic force qE), the obstacle array prevents particles emerging from gap A and diffusing to the left (1) from reaching gap B^-, but "ratchets" particles diffusing to the right (2) to gap B+. Therefore small particles (fast diffusers) move preferentially to the right. [14]-[16] (b) Fluorescence micrograph of separation of 48.5 kb (labeled "b") and 164 kb DNA ("a") using a Brownian ratchet array. Electrophoretograms are measured at 3 mm, 6 mm, 9 mm and 12 mm from the injection point. [16]

These arrays were implemented using the microchannel boundary approach [10] described earlier to insure a vertical flow field. When the flow speed is high (>4 μm/s), no separation occurs because of insufficient time for diffusion. By lowering the flow speed to ~1.5 μm/s and using an optimized orientation of the array with respect to the flow direction to increase the effective diffusion [16], the separation performance (Fig. 4b) is similar to conventional PFGE. However, because of its microfluidic format, these ratchet arrays can easily be integrated into an on-chip total analysis system.

V. Near-Field Molecular Scanners

In the fractionation chips described above, the length of the DNA molecules was not directly measured because intramolecular forces cause the DNA molecule to fold back on itself. A 100 kb molecule with a total length of 64 μm generally occupies a spherical volume with a radius of only ~1 μm, corresponding to the radius of gyration. If one could indeed fully linearize the DNA, one should not only be able to directly measure its length, but could in principle directly observe the location of protein molecules bound to the DNA. Such a scanning ability would be a great aid in unraveling basic questions such as the nature of transcription networks.

A platform for scanning DNA with high resolution consists of a narrow channel through which DNA is driven by electrophoretic forces (Fig. 5) [19], [20]. The posts first uncoil the DNA, and the linearized DNA is then transported into the analysis channel. With appropriate staining, either the DNA or bound molecules can be fluorescently imaged. To improve on the spatial resolution of this method, the pump beam

for fluorescence can be exposed to the channel only through very narrow (e.g. <100 nm) integrated slits, so the DNA is exposed to the pump only through the near field of the evanescent waves penetrating through the slits. The spatial resolution is then not determined by the wavelength of light but by the width of the slit. In practice, the thin slit is made of aluminum, and a microfluidic channel made of silicon dioxide is laid on top of the slit to deliver the molecules. The residence time is translated into molecular length after calibration of the flow speed [20].

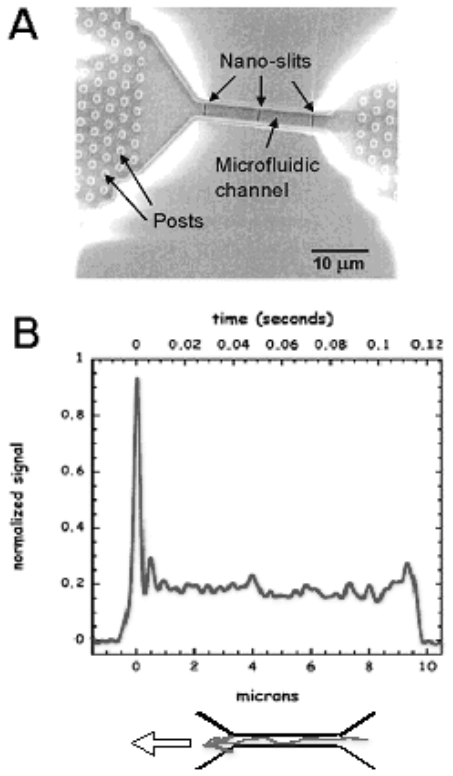

Fig. 5 (a) Scanning electron microscope image of near-field DNA scanner. Posts and microchannel linearize the DNA, and nano-slits localize the near-field excitation of the luminescence probe. (b) Fluorescence signal from stained DNA molecules of 169 kb passing across a slit, showing that the DNA is not fully stretched, especially on the leading end. [20]

Fig. 5b shows the far-field fluorescent intensity vs. time of a 169 kb DNA molecule, confined to a channel width of ~1 μm as it passes over a near-field slit. The DNA molecule has been fluorescently stained over its entire length. The fluorescence rises and falls as the molecule passes over the slit, but the large signal at the leading edge indicates that the leading edge of the DNA is not fully uncoiled (Fig. 5b). Further, one can show that even the extended portion of the DNA is stretched far less than its full length (under 20%) [20]. This results from the fact that the channel is wider than the persistence length of the double-stranded DNA (~50 nm), so that it can begin to recoil and locally fluctuate in shape. This indicates that nano-scale channels are needed.

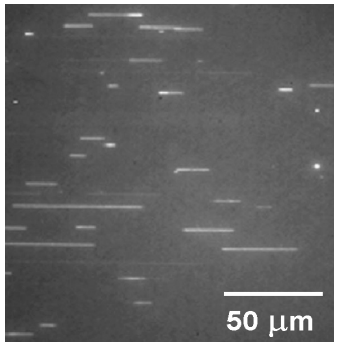

Fig. 6. Fluorescence image of λ-phage DNA concatemers stretched in nanofluidic channels. [18]

VI. Linearization of DNA in Nanochannels

To fully linearize the DNA, we have used nanoimprint lithography [17] to fabricate a series of nanofluidic channels in silicon dioxide, where the channel width is ~50 nm [18]. The mold for the nanoimprint process was created by

interference lithography. DNA molecules can be induced to enter such narrow channels by electrophoresis. Because of the small channel size of the channels, the DNA molecules now cannot coil in the channels and are stretched to much closer to their full length (Fig. 6a) (≥ 75%) [20]. Molecules as large as 500 kb have been successfully linearized. These arrays can clearly be used for rapidly sizing DNA distributions by simple imaging, and are expected to reach their full potential for the high resolution spatial imaging of bound proteins in combination with the near-field scanner design.

VII. CONCLUSION

Micro- and nano-fluidics have been demonstrated to be a powerful tool for the manipulation and analysis of DNA and other biological molecules. These structures can be used to improve existing screening analysis methods, to enable new techniques, and can offer new tools for attacking fundamental questions in biology. Finally, the behavior of biological molecules in such nanostructures poses a great challenge for further understanding of the hard material/soft material interface.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the support of DARPA, NIH and the New Jersey Commission on Science and Technology.

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