Fabrication of Size-Controllable Nanofluidic Channels by Nanoimprinting and Its Application for DNA Stretching

ARTICLE *in* NANO LETTERS · DECEMBER 2003

Impact Factor: 13.59 · DOI: 10.1021/nl034877i

3 AUTHORS, INCLUDING:

Lingjie Jay Guo
University of Michigan
320 PUBLICATIONS **9,191** CITATIONS

SEE PROFILE

NANO LETTERS

2004
Vol. 4, No. 1
69−73

Fabrication of Size-Controllable Nanofluidic Channels by Nanoimprinting and Its Application for DNA Stretching

L. Jay Guo,*,† Xing Cheng,† and Chia-Fu Chou*,‡

Solid-State Electronics Lab, Department of Electrical Engineering and Computer Science, University of Michigan, Ann Arbor, Michigan 48109, and Center for Applied NanoBioscience, Arizona Biodesign Institute, Arizona State University, Tempe, Arizona 85287

Received October 8, 2003; Revised Manuscript Received November 11, 2003

ABSTRACT

We report a new approach to greatly simplify the fabrication of nanofluidic channels with well-controlled dimensions. It is achieved by imprinting a channel template into a thin polymer film cast on a glass cover slip in a *single* step, offering a much higher throughput than previous methods. We demonstrated effective DNA stretching in these nanochannels. This method provides a simple and practical solution for low-cost fabrication of nanofluidic channels, which may serve as a useful tool for chemical analysis system on the nanoscale.

Microfluidic devices have wide applications in liquid handling in biology and chemistry for manipulating samples in small quantities for fast, high-resolution, and low-cost analysis and synthesis.[1,2] In the emerging field of nanobiotechnology,[3−5] further downsizing the fluidic channels to the nanometer scale is attractive for both fundamental studies, such as fluid transport and molecular behavior at extremely small dimensions,[6,7] and in technical applications such as manipulation and high sensitivity detection of single molecules[8,9] for biosensing, chemical analysis, and medical diagnostics. The fundamental challenges imposed on fabricating low-cost nanofluidic channels are to define nanoscale trenches or matrices (templates) and to seal these templates to complete functional nanofluidic devices. The issue of creating low-cost templates has been greatly alleviated due to the advancement of nanoimprint lithography (NIL).[10,11] On the other hand, the sealing technique to close up a nanoscale template is not as accessible as it first sounds. In this paper, we present a practical solution to address this issue and show that the fabrication of nanofluidic channels can be done by simply imprinting a channel template into a thin polymer film cast on a glass cover slip in a single step. Furthermore, it is easy to control the nanochannel dimensions by a simple relationship involving the initial polymer layer thickness and the mold pattern configuration. The use of polymer materials for nanofluidic channels also opens up opportunities for exploiting rich polymer chemistry in nanofluidic applications. We demonstrate one biological application by stretching long genomic DNA in such nanochannel arrays of various dimensions down to 75 nm size. Qualitatively, we found that the degree of DNA stretching is inversely proportional to the channel dimensions due to the confinement effect.

Typical methods of fabricating nanofluidic channels include using electron beam lithography and focused ion beam milling techniques to fabricate high-resolution nanoscale templates, combined with wafer bonding; channel formation by using sacrificial materials; or channel sealing by using poly-dimethylsiloxane (PDMS) polymers.[12] Despite the possibility to create nanochannels by hard bonding, e.g., anodic bonding,[13] of a rigid cover to a substrate that has etched channels, the materials of choice are usually limited to Si and SiO_2. In particular, the high voltage and high temperature used in anodic bonding may exclude its use in low-cost manufacturing process. Nanofluidic channels can also be formed by depositing a sealing material over a sacrificial channel template made of polysilicon[14] or thermal-degradable materials.[15,16] The sacrificial material is then removed by either wet etching or thermal degradation. However, for long fluidic channels with submicron cross sections, it will take an extremely long time, if it is possible at all, to remove the sacrificial materials, thus this technique is not suitable for fabricating large-scale nanofluidic channels. Moreover, the high temperatures used in these two methods preclude the use of most functional polymers due to thermal degradation. On the other hand, there are attempts to use PDMS to seal against nanofluidic channels.[17] The drawback

* Corresponding authors: E-mail: guo@umich.edu and cfchou@asu.edu
† University of Michigan.
‡ Arizona State University.

10.1021/nl034877i CCC: $27.50
Published on Web 12/03/2003

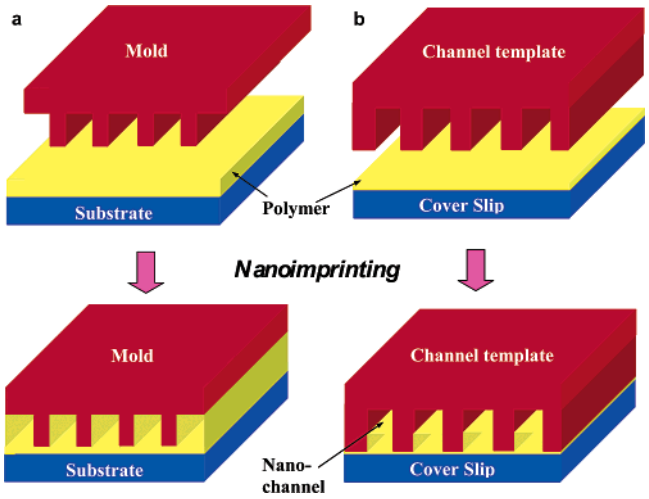

Figure 1. Schematics of (a) the conventional NIL process of using a mold with surface protrusion patterns to imprint into a polymer resist layer and (b) the nanofluidic channel fabrication by using a template mold to imprint into a thin polymer layer to leave unfilled and self-enclosed channels.

of this approach is that the sagging of PDMS material tends to partially or completely seal off the nanochannels due to the rubber-like behavior of PDMS. Recent progress has demonstrated nanofluidic channels fabricated by using a nonuniform film deposition process such as multiple vacuum evaporations or shadow sputtering.[18] Nonetheless, it is a rather intricate and time-consuming process that requires very careful controls of the nonuniform deposition conditions. While this process shows great promise to seal nanoscale channels, it would present a great challenge to seal microscale channels in the same step that is often required to interface the micro- to nanoenvironment.[19]

Our work is aimed at providing a practical solution for fabricating nanofluidic channels that may find applications in nanobiotechnology. The process is inspired by the nanoimprint lithography (NIL) technology developed by the Chou group.[10,11] NIL is a mechanical molding process for nanopatterning by using a mold with nanoscale protrusion patterns on its surface. A schematic of the NIL process is shown in Figure 1a, where the mold protrusions are imprinted into a polymer layer on a substrate at elevated temperature and pressure. To achieve faithful pattern replication, the polymer layer should have sufficient thickness to completely fill the trench region on the mold while leaving a thin residual layer underneath the protrusions. We modified this process to fabricate sealed nanochannels. The process can be explained by Figure 1b: if a very thin polymer layer is used during imprinting, the displaced polymer will not be able to completely fill the trenches on the mold, thereby creating enclosed nanochannel features. In this case, the trench pattern on the mold serves as a channel template, which itself is fabricated by using NIL and reactive ion dry etching techniques. Because NIL is capable of patterning feature sizes down to 10 nm,[11] one can control the lateral dimension of the nanochannels with high precision. The fabrication process can also be well controlled to give predictable channel heights. Consider a layout of a periodic array of channel templates (i.e., gratings) with dimensions shown in Figure 2a. Simple geometrical argument shows that the height of the enclosed nanochannel can be determined by the depth of the etched channel template as well as by the initial thickness of the polymer layer, which follows a simple linear relationship (Figure 2b). As can be seen in Figure 2b, the height can also be controlled by adjusting the ratio of the

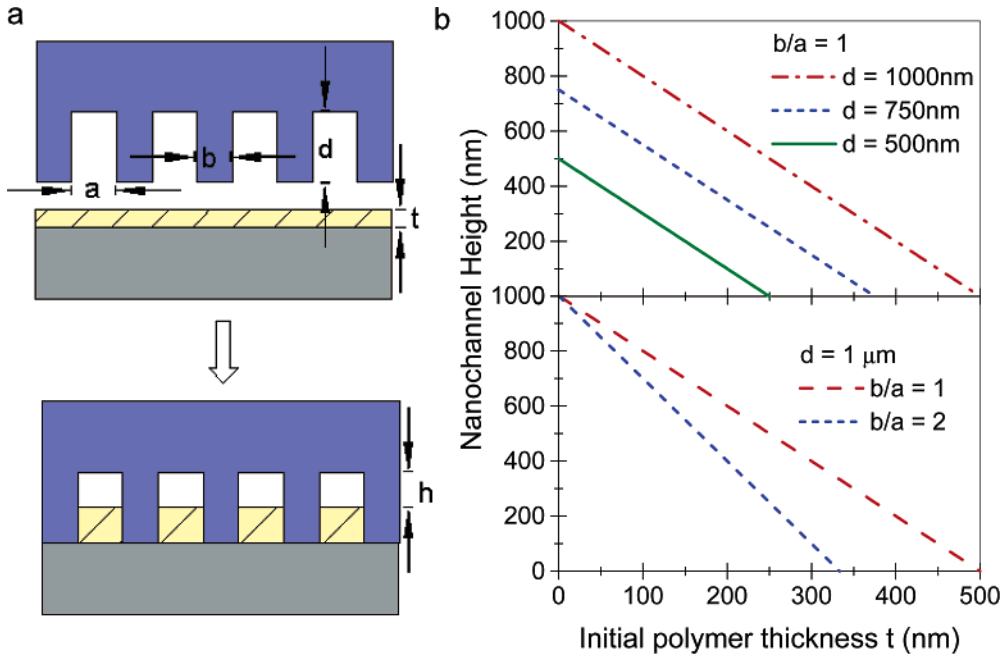

Figure 2. Nanochannel dimension control by varying initial polymer layer thickness and mold pattern configuration. (a) Illustration of the key dimensional parameters for an arrayed channel template: *a*, trench width; *b*, ridge width; *d*, trench depth; *t*, initial thickness of the polymer layer; *h*, nanochannel height after NIL. (b) The simple relationship of the height of the enclosed nanochannels with the initial polymer thickness and the mold pattern sizes, $h = d - (1 + b/a)t$ (obtained by considering the polymer displacement during the imprint process, assuming the polymer material to be incompressible).

ridge width to the trench width on the channel template. These simple relationships can serve as a guideline in choosing the parameters for nanochannel fabrication.

To demonstrate the effectiveness of this method of forming enclosed nanofluidic channels, we first fabricated a template having an array of 300 nm wide and 1 micron deep channels, and the ratio of the ridge width to the trench width is about 4:3. Such a channel template was fabricated on a thermal oxide layer on a Si substrate or glass substrate by using a combination of NIL patterning, and standard metal deposition, lift-off, and dry etching of SiO_2 with the patterned metal mask. This template was used as a mold to imprint into a 220 nm thick poly(methyl-methacrylate) (PMMA) that was spin-coated on a standard microscope cover slip. PMMA was selected not only because it is a commonly used polymer resist in NIL but also because of its optical clarity and low auto-fluorescent background that will facilitate fluorescent microscopy experiments. Note that the process developed here is equally applicable to many other polymeric materials other than PMMA. To guarantee that the oxide surface is hydrophilic, we cleaned the channel template using a solution mixture of $NH_4OH/H_2O_2/H_2O = 1:1:5$ volume ratio for 10 min at 70 °C. To impart surface hydrophilicity to the PMMA film, an oxygen plasma process may be applied to PMMA before channel sealing by imprinting. The imprinting was done at 175 °C and 50 kg/cm^2 (4.9×10^6 Pa) for 5 min, identical to that used in NIL. The template and the glass cover slip were then cooled to ambient temperature and the sealed channels were achieved. Note the processing time is 1−3 orders of magnitude improvement over previously reported methods.[14−16,18] A good and uniform seal of the nanochannel template by the PMMA polymer was verified by scanning electron microscopy (SEM). For SEM characterization, we removed the cover slip in order to cleave the PMMA-sealed nanochannels to expose its cross sections. Cover slip removal can be assisted by using a releasing layer on the glass before coating the PMMA polymer.

To demonstrate the easy control of the channel dimensions, we fabricated several other channel templates having an array of 300 and 100 nm wide channels with various depths. In the second example, we fabricated a channel template with the same ridge-to-trench ratio as in the first example but have a trench depth of 260 nm. The initial PMMA thickness on the cover glass was chosen to be ∼50 nm. After imprinting, a uniform array of channels with a height less than 140 nm was obtained (Figure 3b). It can be seen from the SEM picture that the top of the channel is curved, which corresponds to the meniscus of the PMMA melts during imprinting at 175 °C. Due to this meniscus, the channel height near the sidewalls of the trench reduced to less than 100 nm. Figure 3c shows yet another example where a channel template of 75 nm wide and 160 nm deep trenches (ratio of ridge to trench width ∼3:5) were imprinted into a ∼15 nm thick PMMA on a cover slip, and enclosed nanochannels with height of 120 nm were achieved. We observed that the channel dimensions in all these examples follow remarkably well the simple relationships illustrated in Figure 2.

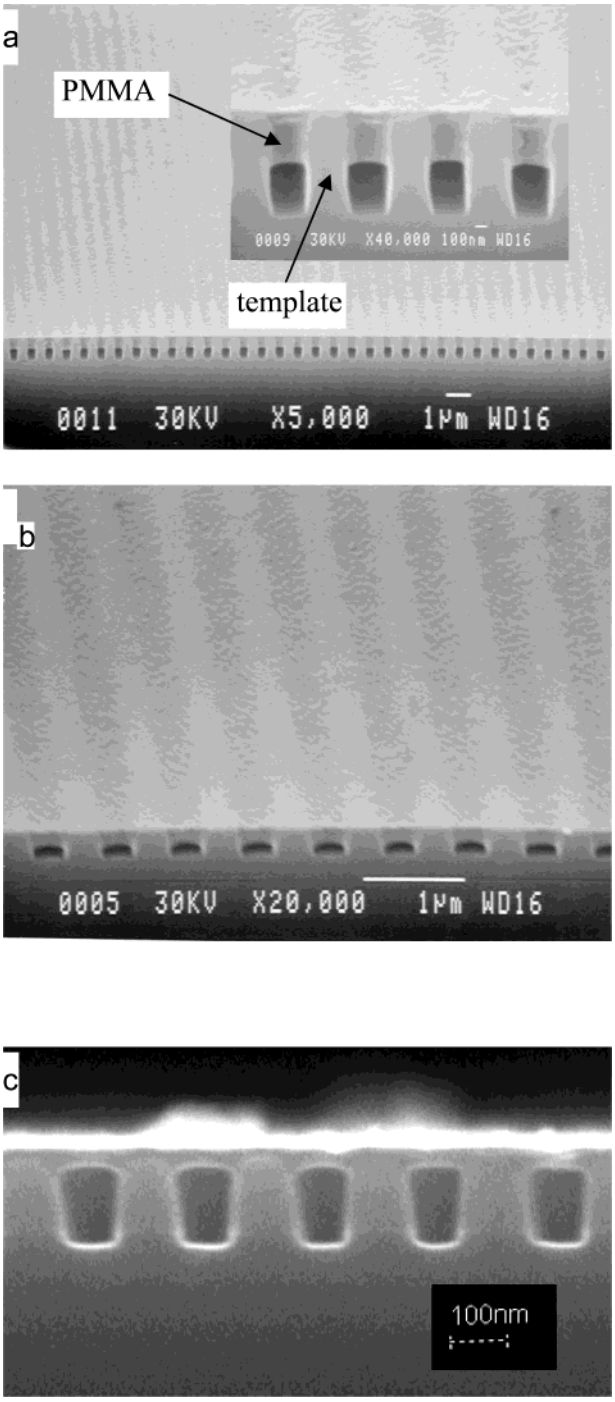

Figure 3. SEM micrographs of imprinted nanofluidic channels of various cross sections. (a) 300 (width) × 500 nm (height) channels; inset shows a close-up view. (b) 300 × 140 nm channels. (c) 75 × 120 nm channels. These fabricated nanochannels follow closely the linear relationship depicted in Figure 2b.

For biological applications, the interaction of biopolymers, such as DNA molecules, with nanostructured channels having dimensions close to the persistence length of the molecule, ∼50 nm in aqueous buffer solutions,[20] provides an entirely new way of detecting, analyzing, and separating these biomolecules.[6,21,22] Consider a 100 kilobase (kb) double-stranded DNA molecule with a contour length of 34 μm (0.34 nm/base pair): the molecule assumes a compact random coil configuration in its natural state with a radius of gyration

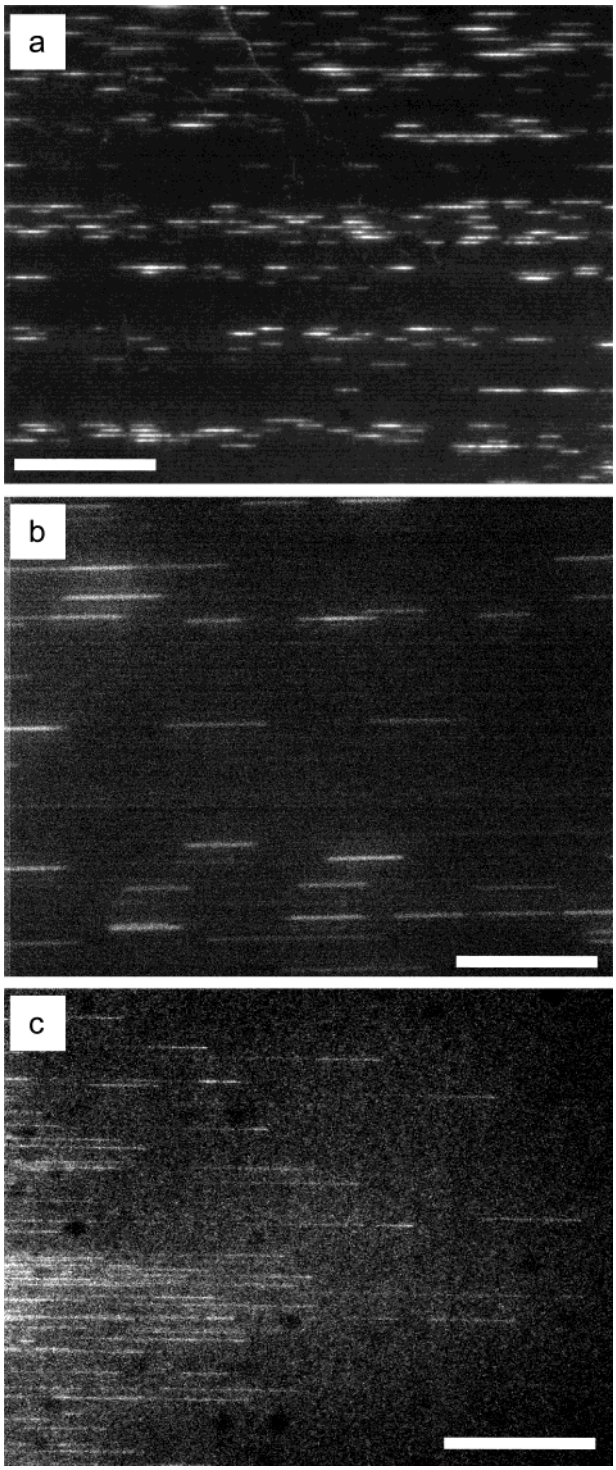

Figure 4. Fluorescent images showing the stretching of 103 kb long T5 phage DNA in the imprinted nanofluidic channels. (a) In 300 nm wide and 700 nm high channels, DNA is stretched but in a lesser degree (estimated to be 15% of its contour length); some overlapping of DNA molecules is observable. (b) In the same chip as in Figure 3a, DNA are more stretched (30%). (c) In the same chip as in Figure 3c, DNA stretching reaches about 95%, and in some cases ~100%. The scale bar is 20 μm in a and b, 50 μm in c. In estimating the degree of stretching, we have taken account of the elongation effect on the DNA backbone due to the labeling of YOYO-1 intercalating dye (see text).

0.47 μm.[23] If such a DNA molecule flows through a microfluidic channel with a cross section much larger than the size of the random coil, the DNA molecule can pass easily while preserving its random coil configuration. On the other hand, if such a DNA molecule is forced to flow through a nanofluidic channel with a cross section comparable to the persistence length of the molecule, it is energetically more favorable for the DNA molecules to be in the stretched states. In this case, the bending of DNA to form a loop would cost energy much higher than the thermal energy.[24] Therefore, nanofluidic channels may be used to stretch DNA, allowing the study of the statics as well as the dynamics of DNA molecules in confined geometries.[25] This could lead to important biological applications, such as quick mapping of restriction-cut genomic DNA segments in very short time (~ minutes), as compared with the hours to days timeframe required in conventional pulsed field gel-electrophoresis.[26] It would also reduce the DNA sample amount required from approximately nanogram to approximately femtogram scale (genomic DNA material in a single cell). In addition, it is easy to make the analysis automatic and multiplexed by using this method.

Table 1. Channel Cross-Sectional Dimension and the Corresponding DNA Stretching Length Obtained by Fluorescent Microscopy (average length and standard deviation)[a]

channel dimension	stretched DNA length	stretching
300 nm × 700 nm	6.2 ± 1.3 um	15%
300 nm × 500 nm	12.7 ± 4.5 um	30%
75 nm × 120 nm	39.8 ± 7.7 um	95%

[a] The percentage of stretching is calculated by dividing the observed average length by the contour length of dye-labeled T5 DNA (42 μm). Some overlapped DNA molecules are excluded in the analysis.

We conducted an experiment of DNA stretching using the fabricated nanofluidic channels. T5 phage DNA (Sigma, St. Louis, MO) of 103 kb (contour length 35 μm) is labeled with YOYO-1 fluorescent dyes (Molecular Probes, Eugene, OR) in a dye/base-pair ratio of 1:5 in 0.5× TBE buffer (45 mM Tris base, 45 mM boric acid, 1 mM EDTA, pH 8.0). DNA molecules were transported into the nanochannels by capillary force. The images were captured by a cooled-CCD camera (Hamamatsu Orca-ER) mounted on an upright fluorescent microscope (Leica DMR) with a 63× oil-immersion objective (N.A. 1.4). Typical images are shown in Figure 4. In nanochannels with cross section of 700 nm by 300 nm, the average stretched length of DNA is 6.2 μm. Compared with the contour length of completed stretched T5 DNA, this corresponds to about 15% of stretching (Figure 4a). When the channel height is reduced to 500 nm while keeping the same channel width of 300 nm, an average of 30% stretching was achieved (Figure 4b). Furthermore, we observed more than 90%, and in some cases ~100%, stretching when these molecules are subjected in 120 nm × 75 nm channels (Figure 4c). These data are summarized in Table 1. The level of stretching was surprisingly effective in such channels. Qualitatively, our experiments showed that the DNA configurations, in this case the degree of stretching, can be altered by reducing the entropic energy of DNA in confined environments. Note that in estimating the degree of stretching, we have taken account of the elongation effect

on the DNA backbone due to the labeling of YOYO-1 intercalating dye. Using the dye/base-pair ratio of 1:5, the contour length is estimated to increase 20% to a value of 42 μm.[7,27] We assume that the dye increases the persistence length by the same factor as the contour length, to a value of ~60 nm. In the 75 nm wide channels used in Figure 4c, the confinement of DNA to two dimensions affects the conformational statistics;[7] this may increase the persistence length by a factor of 2 to ~120 nm, which is comparable to the height but less than the width of the channel. We attribute our observation of ~100% stretching of DNA in Figure 4c to the two-dimensional confinement effect.

We have shown that densely packed arrays of nanofluidic channels with dimensions of several hundreds down to sub-100 nanometers can be successfully fabricated by a modified NIL method. The size of the fluidic channels can be easily controlled by the width and the depth of the channels on the template, and by the initial polymer thickness on the cover slip. These parameters can be varied for fluidic channels of both large and small dimensions for different applications. Particularly, our method is favorable over the nonuniform film deposition process[18] to seal devices fabricated by diffraction gradient lithography[19] to facilitate the interface from micro- to nanoenvironment. By combining with a reverse imprinting technique that we recently developed, it is also possible to build up 3-D fluidic networks.[28] This fabrication method can be extended one step further to develop all-polymer-based nanofluidic channels that can be achieved solely by the nanoimprinting process, leading to a very low cost and high-throughput fabrication of nanofluidic devices. Note that, similar to NIL, this process is susceptible to particles that are present in the processing environment. If there is a particle between the channel template and the PMMA layer, a defect will be generated during the imprinting process. For this reason, all of our processes were carried out in a class-100 cleanroom, which effectively avoided the particle contamination problems. Moreover, making a large array of nanochannels (e.g., > 10^3 as in our sample) is also a practical solution to the potential particle defect problem with the help of built-in redundancy. This method may find its use in integrated genomic, proteomic, and chemical analysis systems.

Acknowledgment. We thank R. Larson and F. Zenhausern for helpful discussions. This work was supported in part by NSF, DARPA, and the Arizona Prop 301 initiative (ANBC).

References

(1) Beebe, D. J.; Mensing, G. A.; Walker, G. M. *Annu. Rev. Biomed. Eng.* **2002**, *4*, 261.
(2) Thorsen, T.; Maerkl, S. J.; Quake, S. R. *Science* **2002**, *298*, 580.
(3) Li, J.; Gershow, M.; Stein, D.; Brandin, E.; Golovchenko, J. A. *Nature Mater.* **2003**, *2*, 611.
(4) Storm, A. J.; Chen, J. H.; Ling, X. S.; Zandbergen, H. W.; Dekker, C. *Nature Mater.* **2003**, *2*, 537.
(5) Craighead, H. G. *Science* **2000**, *290*, 1532.
(6) Turner, S. W. P.; Cabodi, M.; Craighead, H. G. *Phys. Rev. Lett.* **2002**, *88*, 128103.
(7) Bakajin, O. B.; Duke, T. A. J.; Chou, C.-F.; Chan, S. S.; Austin, R. H.; Cox, E. C. *Phys. Rev. Lett.* **1998**, *80*, 2737.
(8) Levene, M. J.; Korlach, J.; Turner, S. W.; Foquet, M.; Craighead, H. G.; Webb, W. W. *Science* **2003**, *299*, 682.
(9) Tegenfeldt, J. O.; Bakajin, O.; Chou, C.-F.; Chan, S.; Austin, R. H.; Chan, E.; Duke, T.; Cox, E. C. *Phys. Rev. Lett.* **2001**, *86*, 1378.
(10) Chou, S. Y.; Krauss, P. R.; Renstrom, P. J. *Science* **1996**, *272*, 85.
(11) Chou, S. Y.; Krauss, P. R.; Zhang, W.; Guo, L.; Zhuang, L. *J. Vac. Sci. Technol. B* **1997**, *15*, 2897.
(12) McDonald, J. C.; Duffy, D. C.; Anderson, J. R.; Chiu, D. T.; Wu, H.; Schueller, O. J. A.; Whitesides, G. M. *Electrophoresis* **2000**, *21*, 27.
(13) Stjernstrom, M.; Roeraade, J. *J. Micromech. Microeng.* **1998**, *8*, 33.
(14) Turner, S. W.; Perez, A. M.; Lopez, A.; Craighead, H. G. *J. Vac. Sci. Technol. B* **1998**, *16*, 3835.
(15) Li, W.; Tegenfeldt, J. O.; Chen, L.; Austin, R. H.; Chou, S. Y.; Kohl, P. A.; Krotine, J.; Sturm, J. C. *Nanotechnology* **2003**, *14*, 578.
(16) Harnett, C. K.; Coates, G. W.; Craighead, H. G. *J. Vac. Sci. Technol. B* **2001**, *19*, 2842.
(17) Chou, H.-P.; Spence, C.; Scherer, A.; Quake, S. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 11.
(18) Cao, H.; Yu, Z.; Wang, J.; Tegenfeldt, J. O.; Austin, R. H.; Chen, E.; Wu, W.; Chou, S. Y. *Appl. Phys. Lett.* **2002**, *81*, 174.
(19) Cao, H.; Tegenfeldt, J. O.; Austin, R. H.; Chou, S. Y. *Appl. Phys. Lett.* **2002**, *81*, 3058.
(20) Bustamante, C.; Marko, J. F.; Siggia, E. D.; Smith, S. *Science* **1994**, *265*, 1599.
(21) Chou, C.-F.; Austin, R. H.; Bakajin, O.; Tegenfeldt, J. O.; Castelino, J. A.; Chan, S. S.; Cox, E. C.; Craighead, H.; Darnton, N.; Duke, T.; Han, J.; Turner, S. *Electrophoresis* **1999**, *21*, 81.
(22) Han, J.; Craighead, H. G. *Science* **2000**, *288*, 1026.
(23) Doi, M. *Introduction to Polymer Physics*; Oxford University Press: Oxford, 1996.
(24) Daune, M. *Molecular Biophysics*; Oxford University Press: Oxford, 1999.
(25) Austin, R. H.; Brody, J. P.; Cox, E. C.; Duke, T.; Volkmuth, W. in *Phys. Today* **1997**, *50*, 32.
(26) Lai, E. *Electrophoresis of Large DNA Molecules: Theory and Applications*; Cold Spring Harbor Laboratory: New York, 1991.
(27) Perkins, T. T.; Smith, D. E.; Larson, R. G.; Chu, S. *Science* **1995**, *268*, 83.
(28) Bao, L.-R.; Cheng, X.; Huang, X. D.; Guo, L. J.; Pang, S. W.; Yee, A. F. *J. Vac. Sci. Technol. B* **2002**, *20*, 2881.

NL034877I