

Gradient nanostructures for interfacing microfluidics and nanofluidics

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(Received 30 April 2002; accepted 26 August 2002)

It is difficult to introduce long genomic DNA molecules into nanometer scale fluidic channels directly from the macroscale world because of the steep entropic barrier caused by necessary stretching of the polymer. We present a very simple technique using optical lithography to fabricate continuous spatial gradient structures which smoothly narrow the cross section of a volume from the micron to the nanometer length scale, greatly reducing the local entropic barrier to nanochannel entry. This technique, diffraction gradient lithography, can be very valuable for the fabrication of micro/nano total analysis systems. © 2002 American Institute of Physics. [DOI: 10.1063/1.1515115]

Nanofabrication of extremely small fluidic structures provides powerful tools for the field of bionanotechnology. They can be used for the direct manipulation and analysis of biomolecules such as stretching genomic DNA in extremely small nanochannels and scanning for bound transcription factors.¹ However, a linear polymer forms a compact random coil in free solution. It is thermodynamically unfavorable for long biopolymers to spontaneously elongate and enter nanochannels directly from the environment due to the large free energy needed to reduce entropy. New insights of understanding the confinement-mediated entropic behavior of biopolymers in ultrasmall nanoscale fluidics have just started to emerge.^{2,3}

In order to uniformly stretch long DNA, the dimensions of nanofluidic structures should be smaller than the persistence length of double stranded DNA (50–70 nm).⁴ We have fabricated arrays of millions of nanochannels over a 100 mm wafer using nanoimprinting lithography (NIL) to stretch, align and analyze long genomic DNA in a highly parallel fashion,⁵ and the resulting sealed channels have a cross section as small as 10 nm × 50 nm. However, it is challenging to move long DNA molecules into the small channels efficiently. For instance, a double stranded T4 phage DNA molecule with a length of 169 kilobases will form a random coil with a radius of gyration approximately 700 nm in aqueous buffer solution;⁴ this is almost 20 times bigger than the opening of the nanochannels. Consequently, problems such as DNA clogging at the junction of the nano- and macroenvironment have arisen and undermined the performance of the nanofluidic devices [Fig. 1(a)]. One solution is to fabricate a micropost array in front of the nanochannels to pre-stretch long DNA molecules before they enter the nanochannels, yet this did not solve the problem completely.

A gradient fluidic structure ranging from micro- to nanometer scales, interfacing the micropost array and the nanochannels, would be desirable to more effectively stretch long linear biopolymers such as DNA before they finally

approach the nanochannels, as schematically illustrated in Fig. 1(b). Imagine that the DNA random coil confinement is slowly increased as one moves towards the nanochannels. Although the net change, $T\Delta S$ due to movement of a DNA molecule is independent of the path, the loss of entropic free energy/length, TdS/dx can be decreased by making the slope small. This retarding force F_e can thus be made arbitrarily small by designing a gradually confining environment. The gradient structure region reduces the steepness of the entropy barrier before DNA molecules finally enter the nanochannels, fully stretched and aligned. This is analogous to a biker, who instead of riding up a steep hill directly, weaves in an S-shaped path so that the local slope is decreased. The problem is how to fabricate such a structure easily and inexpensively. Fabrication of continuous gradient structures can be done using e-beam lithography, which has the flexibility to write any pattern down to a few nanometers, but e-beam lithography has low throughput and is prohibitively expensive for this purpose. For conventional photolithography fabricating features below a few hundred nanometers is challenging.

We have developed a modified photolithography, diffraction gradient lithography (DGL), to fabricate the microposts array and the interfacing gradient structures in one step simply by adding one blocking mask positioned above the photomask in a conventional photolithography setup. Large arrays of nanochannels were first fabricated on an entire Si substrate chip using nanoimprinting lithography, described previously.⁶ This chip was spin coated with positive tone photoresist (AZ5214-E) using standard protocol at 4000 rpm for 1 min after HMDS treatment and baked at 110 °C for 2 min. As shown schematically in Fig. 2, a regular photomask with a uniform micron feature size post array was used to pattern the DNA sample pre-stretching area. A blocking mask, in our case a piece of aluminum foil placed on top of the photomask, served two functions: to mask/protect the area where nanochannels would be kept and to cause light diffraction along the edge of the blocking mask. The distance between the blocking mask and the photoresist surface was about 3 mm. The chip was then exposed by UV light in hard

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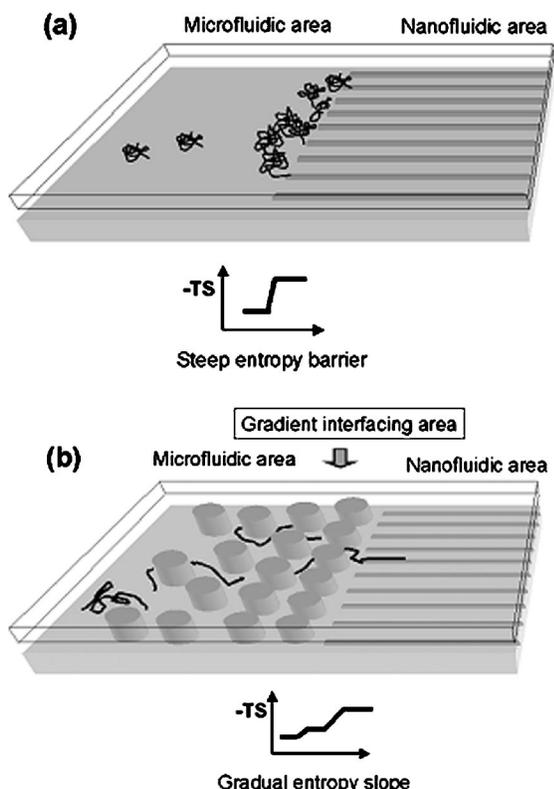


FIG. 1. (a) Schematic diagram showing nanofluidic channels fabricated on the right part of the chip and then sealed by another planar chip (wire frame) to form the enclosed channels. When long DNA molecules are drawn by hydrodynamic flow or electric field from a high-entropy area to confining nanochannels, the molecules tend to stick at the entrances of the channels due to the steep entropy barrier shown on the curve. (b) A schematic diagram of the principle idea showing micropost arrays and an interfacing region of fluidic structures with gradient dimensions designed to pre-stretch DNA molecules before entering nanochannels, resulting in a gradually reduced entropy and less steep barrier curve.

contact mode for 35 s and developed with a standard procedure (AZ312 MIF: H₂O 1:1). Light diffraction caused by the edge of the blocking mask generates a gradient of the light intensity that is cast on the surface of the relatively low contrast photoresist we used, consequently a gradient in the dissolution rate of the resist by the developer.⁷ Figure 2 illustrates schematically the profile of the photoresist after development; notice that all exposed photoresist should be gone except along the light diffraction area but there is a gradient in the thickness of the undeveloped residual photoresist due to the exposure to the diffracted light. The photoresist was used as etching mask during the subsequent reactive ion etching (RIE) process and the gradient patterns in the photoresist were transferred into the underlying Si substrate. A Karl Süss MA-6 contact aligner was used for the DGL process.

Figure 3(a) shows a top view optical image of the actual gradient chip right after photoresist development. The gaps between posts were then etched into the chip using a combination of O₂ and CHF₃ plasma followed by removal of the resist using acetone. Figure 3(b) shows a scanning electronic microscope (SEM) image of the interfacing zone with gradient lateral spacing between microposts after pattern transfer. The area directly under the blocking mask (aluminum foil) with the prefabricated nanochannels is well protected from RIE by the masking photoresist. Since the whole chip sur-

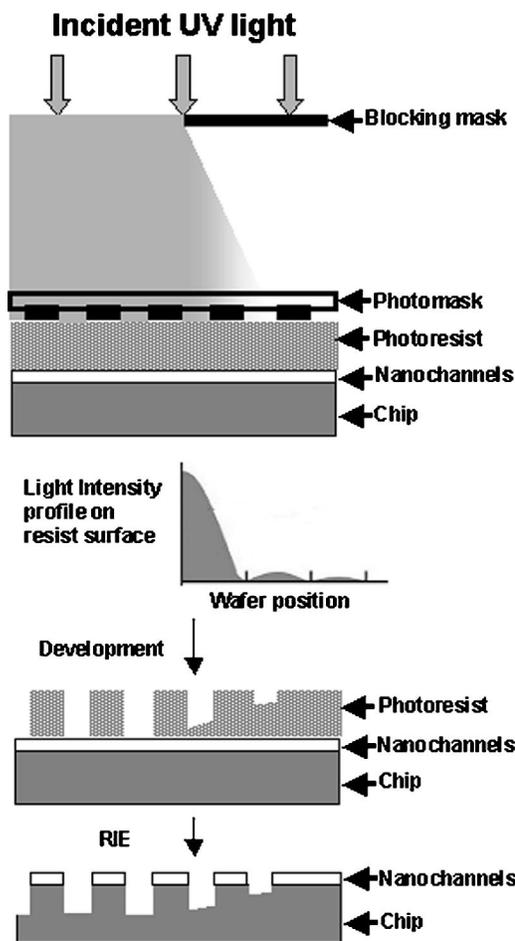


FIG. 2. Schematic illustration of the DGL setup. The substrate chip under the photoresist already has nanochannels on the surface, and was fabricated using NIL.

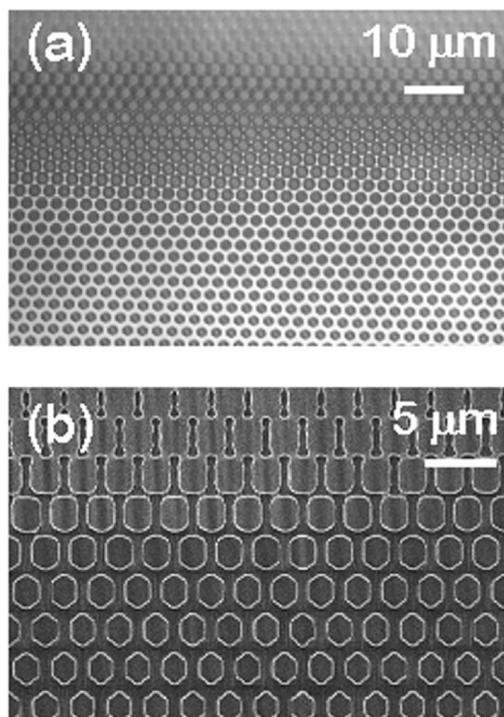


FIG. 3. (a) Optical image of the chip after photoresist development. (b) Top view SEM image of the actual structure after pattern transfer and photoresist removal, showing continuous reduction of the gaps between microposts in the gradient zone.

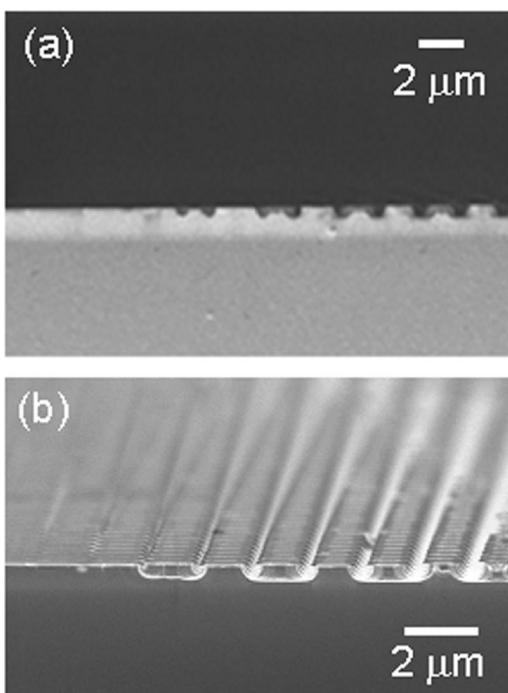


FIG. 4. Two profile view SEM images of the gradient structure area; notice the distance between rows of the microposts is gradually reduced from right to left and the “floor” of the microfluidic chip is continuously elevated from the micropost area on the right side of the image, eventually merging with the nanochannels on the left side, which cannot be seen at this angle. (a), (b) Demonstrated also are the two chips fabricated with two different etching conditions that cause slight variations in the gradient structure dimensions and profiles.

face was covered by prefabricated nanochannels before the DGL process, there were some nanochannels remaining on top of the microposts which cannot be resolved at this magnification.

Light diffraction was used to create gradient patterns laterally as well as vertically. In Fig. 4, cleaved profile SEM images show the gradual reduction of the gaps between the microposts, typically from $1.2\ \mu\text{m}$ gradually to below 400 nm, and the gradual elevation of the “floor” of the fluidic chip to interconnect to the shallower nanofluidic channels. A slight differently etched gradient profile shown in Figs. 4(a) and 4(b) can be controlled by the choice of photoresist, development and etching conditions. The patterns of the gradient zone can be further exploited and controlled using diffraction light simply by adjusting the distance between the blocking mask to the photoresist and the edge patterns of the blocking mask. In our case, we used a straight cut foil edge.

Fluorescently stained long DNA molecules were introduced into both the regular and gradient nanofluidic chips for comparison and charge coupled device (CCD) video images were recorded. In Fig. 5(a), lambda phage DNA entered from the right side of the image, and approached and stalled at the edge of the regular nanofluidic chip, causing fouling of the chip. In Fig. 5(b), DNA molecules were partially uncoiled when they entered the gradient area, and slowed down at the edge of the nanochannels due to “uphill” entropy, however, larger DNA molecules moved into the nanochannels continuously and remained stretched, with significantly improved efficiency. Moving DNA molecules can be seen in the left part of the image as long white streaks after image integration.

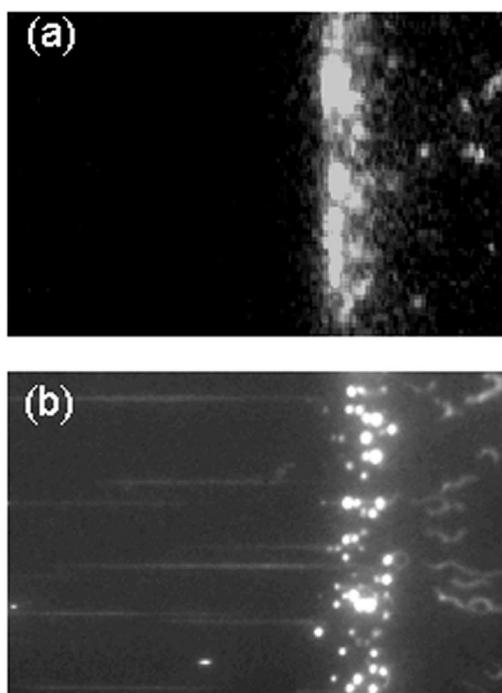


FIG. 5. (a) Intensified CCD images of fluorescent long DNA molecules congregate at the edge of the regular nanofluidic chip without entering the nanochannels in the left part of the image. Individual coiled DNA molecules are visible in the right part of the image. (b) Integrated CCD video images showing partially stretched long DNA molecules in the micropost array and the gradient zone in the right part of the image continuously entering the nanochannels in the left part of the image and being fully stretched.

In summary, we have demonstrated that nanoimprinting lithography can be integrated with conventional photolithography for the fabrication of biomolecular nanofluidic devices. Furthermore, we have taken advantage of the diffraction, often a disadvantageous resolution limiting phenomenon in the semiconductor industry, and turned it into a very simple and flexible technique with which to fabricate the important gradient nanoscale fluidic structures at minimal cost. This process can be easily integrated and automated with current industrial platforms, making it a very valuable processing method in the fabrication of future integrated nanoscale devices.

This work was supported in part by DARPA, NSF and NIH. The authors wish to thank Zhaoning Yu, Wei Wu for the imprinting mold, Paru Deshpande for helping in the etching process, Dr. Lei Chen for developing the imprinting resist, and Dr. Haixiong Ge for imprinting mold surface treatment.

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