Nanofluidic Channels for Biological Manipulation and Analysis

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Abstract

We have used the interface between a nanochannel and a microchannel as a tool for applying controlled forces on a fluorescently labeled deoxyribonucleic acid (DNA) molecule, and observing the molecule in real time. A DNA strand, with a radius of gyration larger than the nanochannel width, that straddles such an interface, is subject to an essentially constant entropic force, which can be balanced against other forces such as the electrophoretic force from an applied electric field. By controlling the applied field we can position the molecule as desired and observe the conformation of the molecule as it stretches, relaxes, and recoils from the nanochannel.

Summary of Research

Fluid-filled channels with dimensions on the order of several tens to several hundreds of nanometers can be used as tools to study fluorescently labeled DNA molecules on an individual basis [1]. A DNA molecule floating in free solution assumes a spherical conformation with a radius of gyration in the range of one-half micron to several microns, depending on the DNA contour length. DNA molecules confined to nanochannels however, are observed to stretch out in one dimension (along the axis of the channel) as depicted in Figure 1. A DNA strand in such an extended state may be interrogated optically, yielding information about the molecule’s length and conformation [2]. In particular, the dynamics of single molecules as they

Figure 1: Schematic of the nanochannel array device. (A) Cross section of device consisting of two bonded fused silica wafers (a, b) with the upper one containing the structure. The microchannel was contacted from the top of the device and fluid reservoirs (c) were attached. Electrical connections to the channel were made by platinum electrodes (d). (B) Close-up of the nanochannel array in the upper wafer. DNA molecules have been drawn in the loading zone (a), as they enter a nanochannel (b), and in an elongated equilibrium conformation in a nanochannel (c).

Figure 2: Time traces of fluorescently labeled DNA molecules confined to nanofluidic channels. Each column of pixels in this image represents the fluorescence intensity along the axis of the channel in one movie frame. Many of these vertical lines are placed side by side to produce the image which shows the position, length, and contour of the DNA molecule in the channel over the course of the entire movie clip. (A) Straightened molecule. First the molecule is driven downward (a) and then upward (b) by 21 V/cm pulses. (B) Molecule with the upper end folded. This molecule is also driven downwards (c) and then upwards (d). The response was the same as in panel A. No unfolding was observed.
contract in the channel or recoil from the channel were studied. In this case, the term “recoil” describes a process of entropically-induced self-extraction from a channel. Further, a distinction was made between molecules that enter the channel straightened out, and those that enter with a front end that happens to be folded over on itself. The part that is looped over on itself is roughly twice as bright as the unlooped portion of the molecule. This is demonstrated by the time trace graphs in Figure 2.

The nanochannel devices, shown in Figure 1, were patterned on a mirror-polished fused silica wafer with a thickness of 500 mm using a combination of electron beam and optical lithography. Initially, a layer of gold, 25 nm thick, was evaporated onto poly(methyl-methacrylate) (PMMA) electron-beam resist to help draw current during the electron-beam process. Negative patterns of the nanochannel regions were exposed using a JBX-9300FS electron beam lithography system. After removal of gold and PMMA development, the patterns were transferred to a chrome mask by evaporation and lift off. Microchannel patterns were then added to the mask using optical lithography and the same lift-off process. Both micro- and nanochannels were etched simultaneously using a Plasmalab 80Plus REI with a CHF$_3$/O$_2$ mixture at 50 Watts for 20 min. Access holes were created by alumina powder blasting from the backside of the wafer. Finally a 170 mm fused silica cover wafer was touch bonded and annealed at 1050°C to the device wafer, enclosing the channels. Nanoports were sealed to the access holes forming buffer reservoirs. Several electron micrographs of the nanostructures before bonding are shown in Figure 3.

Finally, we have modified the microscope setup in order to allow for higher throughput DNA detection. As opposed to illuminating of a large field of view of the chip, and detecting light from that field of view with a charge coupled device (CCD) camera, we now focus two or more laser spots on a channel, as depicted in Figure 4. When a DNA strand passes through a spot, its associated fluorophores are excited and emit a fluorescent signal. Emitted light is collected by one of several aligned optical fibers and detected by avalanche photodiodes. This arrangement allows for molecules to be driven through nanochannels and detected at high speeds. Information regarding velocity, fragment length, and conformation may be collected for thousands of molecules in the course of 1 minute.

References


Figure 3: Electron micrograph of nanochannel array etched into 500 mm fused silica wafer. (A) Top-down view showing the interface between the microchannel and the array of nanochannels. Both were etched 100 nm deep. (B) Entrance to two nanochannels. The channels are 90 nm wide. The wall separating the two channels is 910 nm wide. (C) Closer view of a nanochannel entrance. Floor roughness is 10 x 20 nm and is attributed to the etching process.

Figure 4: Cartoon of an array of nanofluidic channels, with two laser spots focused on one of the channels. DNA molecules may be driven through the channels and detected at high speeds. (a) DNA molecule in a microchannel, (b) DNA partly in a nanochannel, (c) DNA strand elongated in a nanochannel and interaction with the probe volume of laser spot number one.