Fabrication of Nanofluidic Devices for DNA Confinement

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Abstract:

Confining deoxyribose nucleic acid (DNA) in a nanochannel affords the elongation necessary for DNA barcoding, a promising genomic mapping technique. Therefore, our objective was to fabricate nanofluidic devices that would allow for efficient confinement of genomic DNA. To limit DNA sticking to the channel walls, fused silica, an inherently hydrophilic material, was used as the device substrate. After overcoming many fabrication hurdles, these nanofluidic devices were successfully fabricated; however, DNA sticking to the nanochannels proved to be a significant issue during device loading. Scanning electron microscopy (SEM) images of the nanochannels indicated that channel roughness was likely the cause. The fabrication process was further refined, in regards to electron-beam lithography, to minimize this roughness.

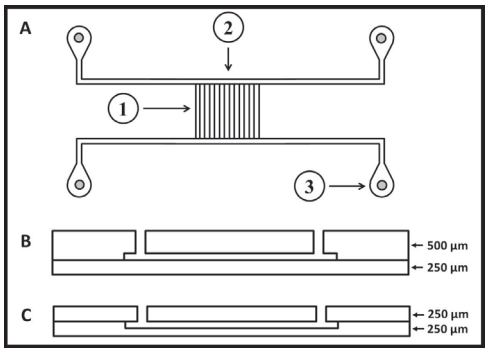

Figure 1: A) Device layout: 1. Nanochannels, 2. Microchannels, 3. Through holes. B) Cross-section of device design. C) Cross-section of initial device design, which did not bond well due to warping.

Introduction:

Accurate, low-cost genome sequencing will have broad implications in a wide variety of fields such as personalized medicine [1]. Next-generation sequencing methods have made tremendous strides over the past decade in accuracy and read length. However, these 'short read' shotgun sequencing methods possess inherent limitations for entire genome sequencing. Repetitive genomic regions, which account for half of the human genome, cannot be directly mapped [2], and technologies that rely on ensemble measurements are unable to detect rare genomic variations [3].

Confining genomic DNA to a nanochannel can afford numerous sequence analysis techniques without the need for amplification or DNA fragmentation. If DNA can be loaded into a channel ~ 50 nm wide, the DNA molecules cannot fold back on themselves and are forced by physical confinement to be in an elongated, linearized state [3]. If the DNA is fluorescently labeled at specific sequences, the confined DNA creates a distinct optical pattern, resembling a barcode that provides a large-scale view of the genomic DNA. This technique known as DNA barcoding provides a scaffold for *de novo* construction of sequence data obtained from next-generation sequencing methods, allowing for entire genome sequencing [1].

Thus the objective of the project was to establish a robust method for fabricating nanofluidic devices that effectively confine genomic DNA. Specifically, we wanted to use fused silica, which is inherently hydrophilic, as the substrate material to limit DNA sticking to the nanochannel walls [3].

Device Fabrication:

The nanofludic devices were fabricated on 10 cm diameter, 500-μm-thick fused silica wafers. Each device contained of an array of nanochannels between two parallel microchannels with reservoirs for loading (Figure 1). To fabricate the nanochannels, electron-beam lithography was used. Since fused silica is an insulating material, 30 nm of aluminum was deposited on the wafers to prevent charging. An electron beam resist layer was spin-coated on top of this aluminum layer, and standard techniques were used to define, develop, and etch the device patterns. This process produced channels 60 nm deep and 50-150 nm wide (depending on the predetermined electron beam exposure level). The microchannels and reservoirs were patterned by contact photolithography and were etched by a wet and dry etch sequence. Sandblasting was then used to establish the through holes on the back of the wafer. After thorough cleaning, the etched wafer was physically pressed onto a 250-μm-thick wafer and fusion-bonded overnight.

Initial attempts to fabricate the devices using two 250-μm-thick wafers (Figure 1) were unsuccessful because it was difficult to establish a good bond between the wafers. Analysis indicated that significant warping in the wafers, most likely due to undesired etching, was the possible cause.

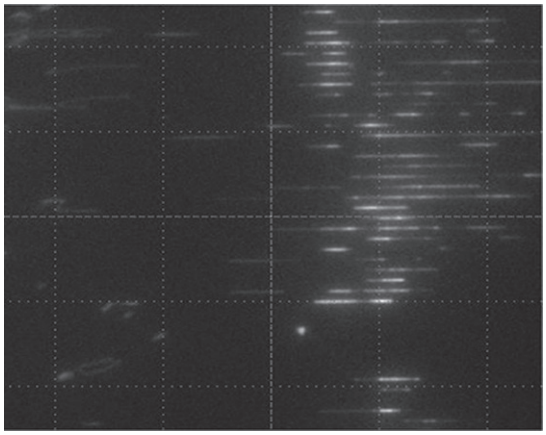

Figure 2: Fluorescently dyed λ-DNA in nanochannels. Bright areas are locations of DNA sticking.

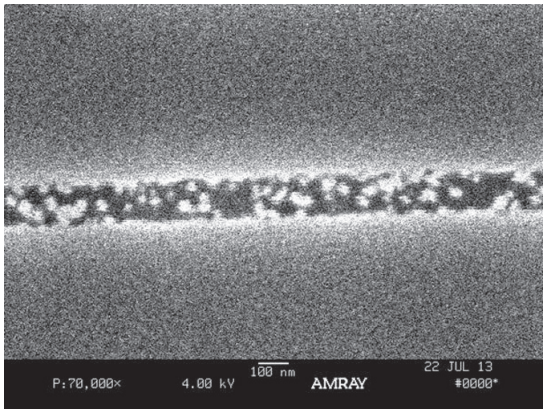

Figure 3: SEM image of channel obtained from initial fabrication procedure.

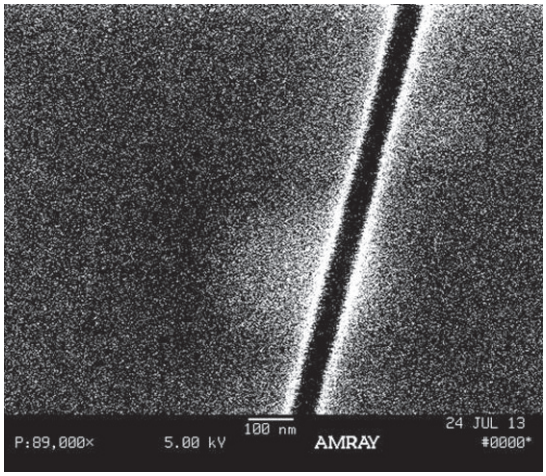

Figure 4: SEM image of channel obtained from modified fabrication procedure.

Device Evaluation:

The device was filled with 2.5X tris, borate, ethylenediaminetetraacetic acid (TBE) aqueous buffer containing B-mercaptoethanol (5% w/w), ascorbic acid (0.07% w/w), and polyvinylpyrrolidone (0.01% w/w). Genomic DNA from virus phage λ, dyed with fluorescent label YOYO-1, was pipetted into the reservoir, pumped through the microchannel, and subsequently forced into the nanochannels by an applied voltage. However, as DNA moved through the nanochannels, a large portion of the DNA was sticking to the channel walls (Figure 2).

SEM micrographs of the device indicated that there was significant roughness on the bottom and sides of the nanochannels which is most likely the reason for the DNA sticking (Figure 3). To reduce channel roughness, the electron-beam lithography process was modified by depositing the 30 nm aluminum layer above the electron beam resist instead of below it. This modification allowed for a more complete removal of the aluminum layer, thereby creating a cleaner fused silica etch. Nanochannels produced following this protocol were significantly smoother (Figure 4); however, more analysis and testing is needed to confirm that channel roughness has been reduced.

Future Work:

After establishing a robust fabrication procedure that produces smooth channel walls, the next step would be to load DNA into the nanochannels to determine if the modified procedure has reduced DNA sticking. These nanofluidic devices will be used to further our understanding of how confined DNA behaves, which will ultimately help enable better genomic mapping technologies.

Acknowledgments:

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References:

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