DNA Electrophoresis in Sparse Ordered Obstacle Arrays

Alyssa Terry

Biomedical Engineering, Mississippi State University

NNIN REU Site: Nanofabrication Center, University of Minnesota-Twin Cities, Minneapolis, MN

NNIN REU Principal Investigator(s): Dr. Kevin Dorfman, Chemical Engineering and Materials Science, University of Minnesota-Twin Cities

NNIN REU Mentor(s): Dr. Jia Ou, Daniel Olson; Chemical Engineering and Materials Science, University of Minnesota-Twin Cities

Contact: abt66@msstate.edu, dorfman@umn.edu, ouxxx023@umn.edu, olso1887@umn.edu

Abstract and Introduction:

Engineering faster methods for deoxyribonucleic acid (DNA) separation is critical for the future of disease diagnosis and forensic work. To date, electrophoresis for DNA separation can only be performed on samples with fewer than around 20,000 base pairs using constant field gel electrophoresis. By increasing the pore size of traditional electrophoresis media, longer DNA with more base pairs can also be separated under a direct current (DC) field in microfluidic devices.

This study focuses on two main challenges: optimizing fabrication methods for creating micro-features on silica using the facilities available in the University of Minnesota fabrication center and understanding the post-collision mechanism of the DNA molecules. To study the DNA collision, first a procedure for creating an ordered hexagonal array in silica with 1 mm features had to be developed. Scanning electron microscopy (SEM) was used to inspect the effectiveness of the fabrication procedure. For the electrophoresis experiments, the DNA was dyed with YOYO-1 and then placed in the chip under an electric field. An inverted microscope controlled by LabVIEW, in addition to MATLAB software, was used to image and analyze the DNA during separation. Using the images recorded from the experiments, the DNA was analyzed for collisions, shape upon impact, velocity and time to relaxation.

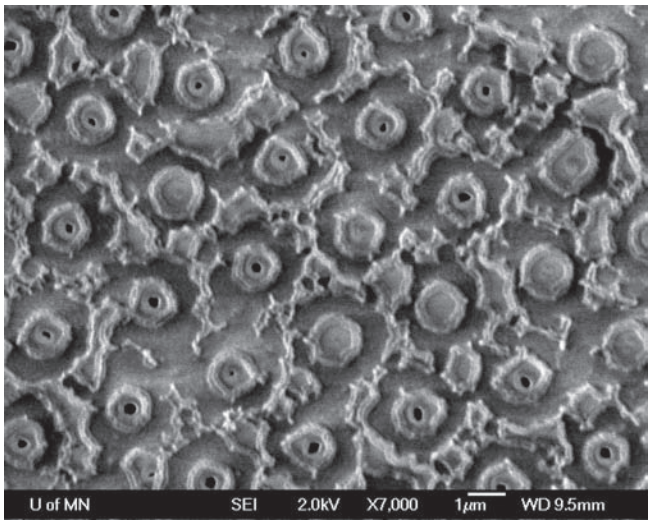

Figure 1: SEM of Method 1.

Device Fabrication:

A device consisting of a channel with posts 1 μm in diameter spaced 3 μm apart was needed to examine DNA collisions during electrophoresis. The chip consisted of a channel with the aforementioned specifications and two ports into which to load the DNA.

Method 1. Wafers were patterned using Shipley 1805 photoresist, exposed using a MA/BA6 contact aligner, and developed using 351 developer. Unwanted photoresist was removed using an oxygen plasma etch on the STS etcher. Posts were produced by etching through the silicon wafer in a deep trench etcher. Then 400 nm of silicon dioxide (SiO_2) was grown on the chips to provide electrical insulation.

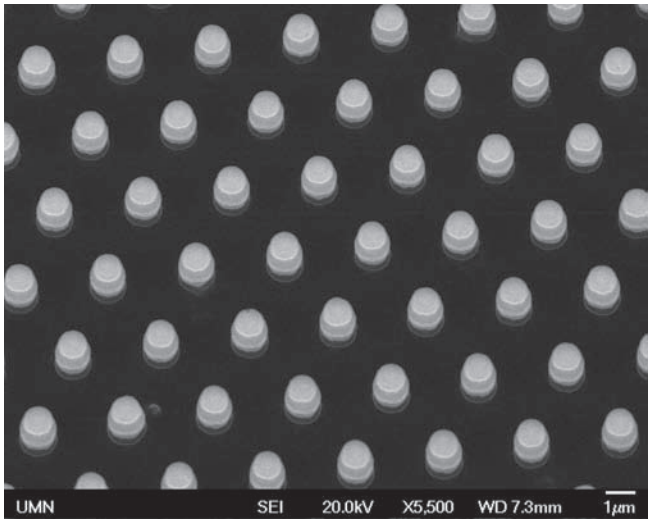

Figure 2: SEM of Method 2.

Method 2. A 25 nm layer of SiO_2 was grown on an Si wafer and then patterned using 0.5 μm of Shipley 1805 photoresist and developed in 351 developer. The wafers were then coated with 300 Å of chromium (Cr) in the e-beam metal evaporator. Acetone was used to remove all of the photoresist, including the photoresist coated with Cr. A pattern of Cr dots remained. Then buffer oxide etch (BOE) was used to etch through the SiO_2 layer, and Cr-125 etch was used to remove the Cr, leaving small posts of SiO_2. Deep trench etching was then used to etch through the Si and create 2 μm high posts. Then 400 nm of SiO_2 was grown on the wafer to provide electrical insulation. After the silicon was patterned, the chips were cut and holes were drilled into them at each of the ports. A glass slide with ports at the same location was adhered to the silicon using an epoxy.

DNA Electrophoresis. Lambda DNA was dyed using YOYO-1. Dyed DNA was loaded into one port on the device and 60 volts applied to the chip to make the DNA move from one side of the channel to the other. DNA was examined using a camera and microscope controlled by LabVIEW and analyzed with MATLAB.

Results and Discussion:

Method 1 produced inconsistently sized and spaced posts. Photoresist remained in the channel, therefore blocking the flow of DNA through the chip. The photoresist may have been a artifact of the photolithography procedure. Figure 1 depicts the final results of Method 1.

As shown in Figure 2, the second method produced consistently sized and spaced posts. No photoresist remained in the channel, and DNA was free to flow between and collide with the posts.

Figure 3 shows the position of a single molecule of DNA through the channel in time. Two collisions are visible: one at ~ 0.75 seconds and another at ~ 1.75 seconds. Velocity was constant as the DNA approached and left a post, but was reduced to 0 frames/sec upon impact.

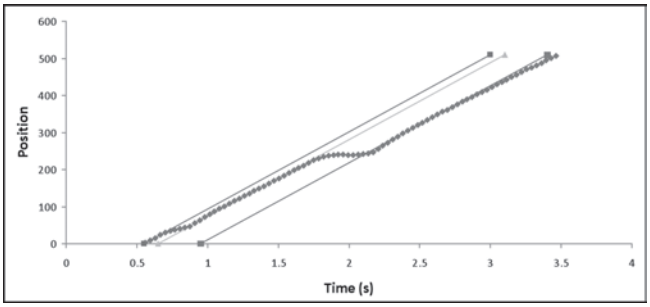

Figure 3: DNA time vs. position through channel.

Conclusions and Future Work:

Method 2 created ordered arrays of posts that can be used effectively for analysis of DNA collisions in electrophoresis. In the future, Method 2 will be used to fabricate chips for DNA separation and collisions.

Acknowledgements:

Thanks to Dr. Kevin Dorfman for this opportunity, Dr. Jia Ou and Dan Olson for their guidance and support, and the National Nanotechnology Infrastructure Network Research Experience for Undergraduates (NNIN REU) Program for funding.

References:

[1] S. A. Campbell, The Science and Engineering of Microelectronic Fabrication, Vol. 2: Oxford University Press, 2001.

[2] M. J. Madou, Fundamentals of Microfabrication: The Science of Miniaturization, 2nd edition. Boca Raton, FL: Taylor & Francis, Inc, 2002.

[3] J. L., Vivoy, "Electrophoresis of DNA and other polyelectrolytes: Physical mechanisms." Reviews of Modern Physics, vol 72, pp 813-872, 2000.