DNA Electrophoresis in Microfabricated Post Arrays

Jason Lee
Chemical Engineering, University of Massachusetts-Amherst

NNIN REU Site: Nanofabrication Center, University of Minnesota-Twin Cities, Minneapolis, MN
NNIN REU Principal Investigator(s): Dr. Kevin Dorfman, Chem.Eng. and Materials Science, University of Minnesota-Twin Cities
NNIN REU Mentor(s): Dan Olson and Dr. Jia Ou, Chem.Eng. and Materials Science, University of Minnesota-Twin Cities
Contact: jasonl@student.umass.edu, dorfman@umn.edu, olso1887@umn.edu, ouxxx023@umn.edu

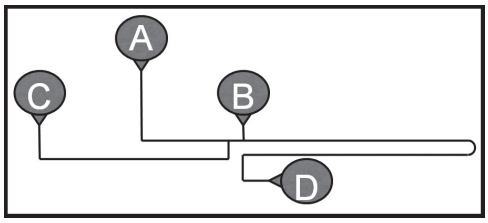

Figure 1: Schematic of dual channel sparse post array device.

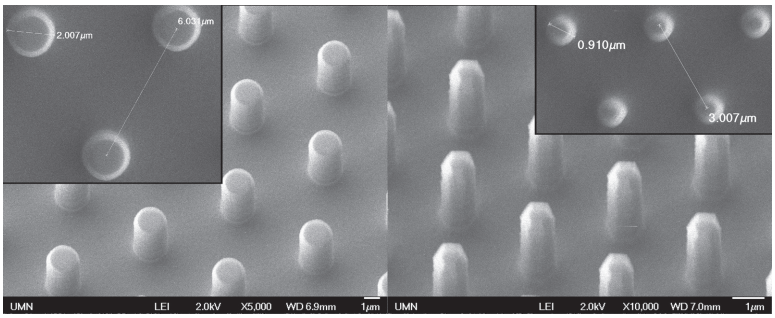

Figure 2: First post array (left) and second post array (right).

Abstract and Introduction:

Agarose and polyacrylamide gel electrophoresis enable researchers to study biological systems through numerous applications that require separating deoxyribonucleic acid (DNA) by size. However under many conditions, gels cannot resolve large DNA unless using pulsed field gel electrophoresis, which turns an hour process into a day. Hexagonal post arrays have been shown to be more efficient. Separation ability depends on post diameter and spacing [1]. The microfluidic device fabricated employs two arrays, the first with 2 μm posts at 6 μm spacing and the second with 1 μm posts at 3 μm spacing, to look for greater separation than either array alone. Rearrangement of the reservoirs (Figure 1, A-D) decreased DNA loading times during experiments. Post fences at reservoirs were added to block debris and improve device lifetime.

Device Fabrication:

A 1000Å layer of silicon dioxide (SiO_2) was grown on cleaned silicon <100> wafers using thermal oxidation. Oxide thickness was confirmed using a Nanospec spectrophotometer. The wafers were primed with hexamethyldisilazane before spinning Shipley 1805 photoresist at 3000 rpm for 60 seconds. Following a one minute, 105°C softbake, the resist was exposed for 4.5 seconds using chrome mask contact lithography. The pattern was inverted in an ammonia image reversal oven followed by 14 minutes of ultraviolet flood exposure. The mask was developed in a 7x dilution of developer 351 for about 25 seconds. A single drop of developer was placed on each fence for four extra minutes for full development. After passing inspection under an optical microscope, wafers were oxygen cleaned for 30 seconds in a reactive ion etcher followed by buffered oxide etching of the dioxide for roughly 150 seconds. The resist was removed with acetone, methanol, isopropyl alcohol, distilled water, and then 10 minutes of oxygen plasma cleaning. Using the silicon dioxide mask, the silicon was etched in the deep trench etcher down to about 4.5μm deep. A P16 profilometer confirmed channel depth and the dioxide was removed with buffered oxide etcher. After inspecting the quality of individual devices, the wafers were spun with Shipley 1818 resist to protect the posts during dicing and reservoir drilling. Devices were then thermally oxidized for 7 hours.

Devices were imaged by scanning electron microscope (SEM) (Figure 2) before being glued to drilled glass back-plates. Glass cover slips were spun with Norland Optical Adhesive 81 (NOA81) and then sandwiched between two flat silanized slabs of polydimethylsiloxane (PDMS). Ultraviolet light cured the adhesive except at the surface of the PDMS to provide a thin film for attaching the cover slip and sealing the channels. Reservoir connectors were glued onto the backplate. Distilled water followed by 5x Tris/Borate/EDTA (TBE) electrophoresis buffer with polyvinylpyrrolidone was pumped into the channel prior to usage.

DNA Electrophoresis:

Lambda DNA, purchased from New England Biolabs, was digested with XhoI restriction enzyme to yield a 15 kilobase and a 33 kilobase fragment. These fragments were mixed with uncut lambda DNA for final concentrations of 24 μg/mL and 8 μg/mL respectively. The DNA was dyed with YOYO-1 and pipetted into reservoir B (Figure 1). Other reservoirs were filled with 5x TBE with 10% dithiothreitol. DNA was pulled from reservoir B towards reservoir C into the shifted-T by setting electric fields. DNA in the shifted-T was injected by setting fields to push from A and pull towards D at 10 V/cm. Fluorescence intensity was measured over time using a photomultiplier tube on an oil immersion lens microscope with a motorized stage. Measurements were made at four locations: immediately before and after each array.

Results and Conclusions:

Two peaks in the intensity vs. time plot showed the first array resolves the three species into two groups (Figure 3). The addition of the second array improves separation, resolving all three species (Figure 4). However, peaks overlap greatly, reducing the measured resolution. The overlap is likely due to DNA dispersion. The U-turn is a likely source of additional dispersion because the DNA on the outer side of the turn must travel farther than the DNA on the inside of the turn, which results in a widening of the DNA band before entering the second array. More trials are necessary to pinpoint the cause of performance loss. In comparison to previous processes that used photoresist or chrome etch masks, this process proved more reliable and cost effective.

Other Processes:

A 2 μm deep channel was made with regular reactive ion etching instead of deep trench etching to eliminate scalloping. From this wafer, a PDMS mold was cast and then silanized. The mold was placed on a cover slip spun with NOA81 and cured similarly to the above process. SEM images show cured NOA81 replicates the posts. This process makes devices for DNA separation studies to be even easier to produce. A new etch recipe is needed to eliminate the undesired sloped profiles.

Acknowledgements:

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References:

[1] Kevin D. Dorfman, "DNA Electrophoresis in Microfabricated Devices," Reviews of Modern Physics (in press).

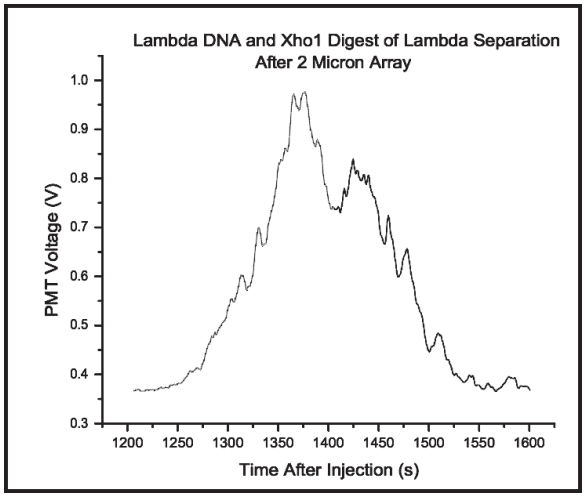

Figure 3: DNA fluorescence intensity over time after the first array.

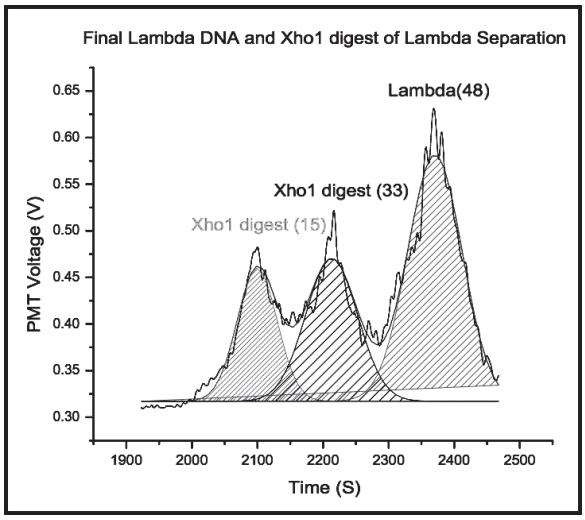

Figure 4: DNA fluorescence intensity over time after both arrays.