Fabrication of Nanochannels for Linearization and Diffusion of DNA

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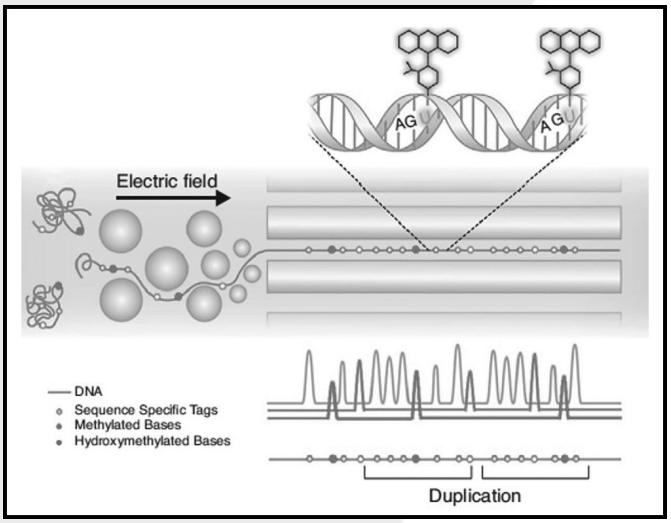


Figure 1: This image from "Beyond sequencing: optical mapping of DNA in the age of nanotechnology and nanoscopy," shows the act of straightening DNA, as well as the optical barcode that can be derived from the fluorescent markers. Reprinted with permission from [1] Levy-Sakin, M. and Ebenstein, Y. (2013). Current Opinion in Biotechnology. 24, 690-698.

Introduction:

Optical mapping of deoxyribonucleic acid (DNA) has emerged as a viable alternative to help with read length restrictions in conventional sequencing. Rather than attempting whole genome sequencing, which often has errors and gaps, optical mapping uses fluorescent imaging of large (~ 10 kilobase pair -1 megabase pair), linearly arranged, individual DNA strands in order to view large scale patterns that would be difficult to obtain by sequencing [1]. As shown in Figure 1, points of interest on the DNA are marked, and a unique barcode characteristic of the features present in the sequence is created. The optical mapping technique requires forcing the DNA to be in a linear state, which isn't preferred, as the polymer has maximum entropy in a random coil state. Nanochannel devices alleviate this problem by confining the DNA molecule to a one dimensional space, where the polymer will have no choice but to exist linearly. The focus of this project was to study the dynamic behavior of an individual, isolated DNA molecule in confinement which can be directly deduced from its diffusion properties in response to the variation of the width of the channel.

Experimental Procedure:

Four-inch fused silica wafers were used as preferred substrate for device fabrication because of their non-positive charge, to avoid sticking of negatively charged DNA backbone, and low autofluorescence, to achieve higher signal to noise ratio as compared to silicon in imaging process.

The basic structure of the device is shown in Figure 2, and consists of four DNA feeder holes (1-4) to pipet DNA dissolved in buffer solution into the device.

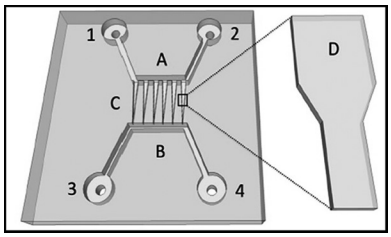


Figure 2: This figure is a basic overview of the device, with the design belonging to Gupta, et al. Reprinted with permission from [2] Gupta, D., et al. (2014). Mixed confinement regimes during equilibrium confinement spectroscopy of DNA. J. Chem. Phys. 140, 214901-214913.

These holes connect to microchannels (A&B) approximately 50 μm in width, which ease the entropic jump between a bulk coil state and a linearly confined nanoscale state. The nanochannel (C&D) region of the device vary in width from wafer to wafer, as the effects of varying confinement widths on the extension and diffusivity of the DNA is desired [2]. However, despite the varying widths of the device channels, each device sought to have the same depth as its width. The nanochannels in the devices were created using electron beam lithography. A conductive layer, usually gold or aluminum, was added on top of the 950 poly(methyl methacrylate) (PMMA) e-beam resist because the substrate was nonconductive. Without a conductive layer, the electron beam spot size would be bigger than desired, leading to lower resolutions. Photolithography with AZ9260 resist was used to create the microchannel and reservoir regions, where the highest possible write resolution is not necessary. After creating patterns on the resist, reactive ion etching was used to etch the patterns into the surface of the substrate. Afterwards, fusion bonding was done at 1000°C to seal the device with a fused silica coverslip.

To study the diffusion of DNA at equilibrium, fluorescence microscopy was employed. Lambda-DNA (New England Biolabs, ~ 48 kbp) marked with YOYO dye was imaged using a laser, and a snapshot was taken every five seconds. It could be assumed that the mass at a given point was correlated to the

intensity of emitted light, as the DNA was labeled with dye in even intervals, so a mass profile based on the light intensity of a given point was created. From this data, the center of mass can be established for each frame, and the movement of the center of mass was calculated. Mean squared displacement (MSD) can be found from the movement of the center of mass, and since MSD = 2Dt for one dimension random walk, the diffusion coefficient D can be found by plotting MSD vs. time, t. Diffusivity was expected to decrease with the confinement size, because of the greater amount of monomer-environment interaction.

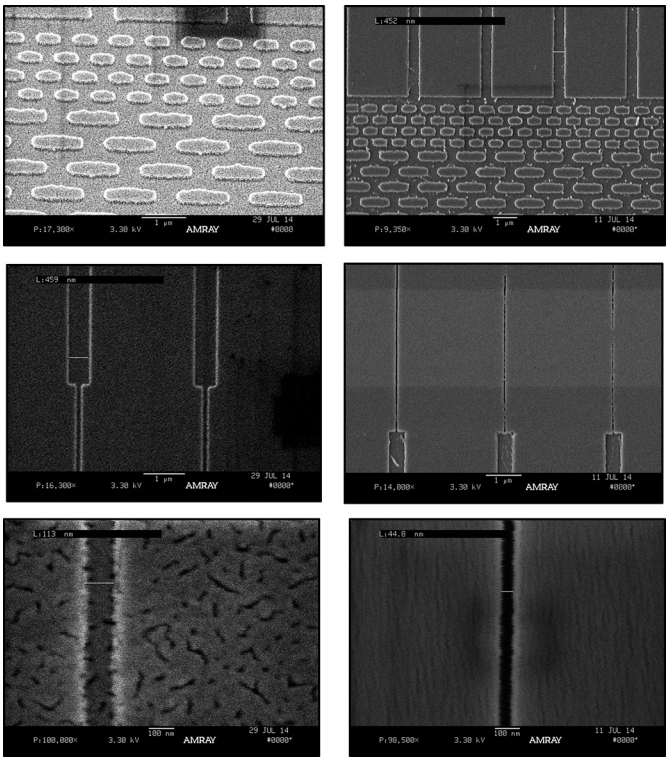
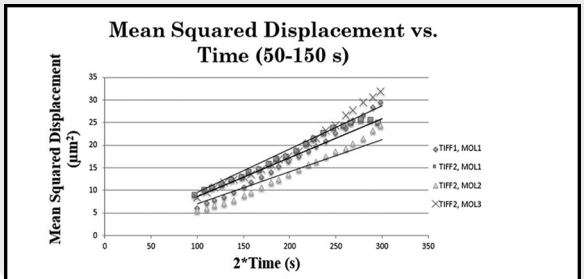


Figure 3: These devices were fabricated over the course of the project, with the nanochannel feature designs from Lam, et al. [3].

Results and Conclusions:

Figure 3 is a collection of scanning electron microscopy images that depict two different devices which were fabricated as a part of this project: a 90 nm device (left column), and a 60 nm device (right column). They show the three main features of the nanochannel region of the device. The top images show the pillar region, a gradient of hexagonally close packed protrusions which help linearize the DNA. They act like the bristles of a comb to untangle the DNA from its bulk state. The middle images show the concentration channels. These act like a funnel and collect the DNA at its interface with the nanochannels (pictured) so that more polymers will be captured in a single image. Finally, the bottom images show the nanochannels where the DNA was confined at equilibrium. Nanochannel region was based on designs used by Lam, et al. [3].



	Slope (μm²/s)	R²
TIFF1, MOL1	0.0958	0.9503
TIFF2, MOL1	0.0862	0.9153
TIFF2, MOL2	0.0865	0.9818
TIFF2, MOL3	0.0709	0.9252
Average	**0.08485±0.005**	0.94315

*Figure 4: This graph was created by plotting the mean squared displacement of four different DNA molecules vs. 2*time while confined to a 100 nm channel.*

Diffusion data was gathered with a 100×100 nm device. Our preliminary diffusion results in Figure 4 agree with the expectation that increased confinement would lead to a lower diffusion coefficient, as the diffusivity of DNA at 100 nm confinement is 0.08485 ± 0.005 $\mu m^2/s$ (E). Comparatively, $D_{bulk} = 0.46 \pm 0.03\ \mu m^2/s$ [4], which is significantly higher. We can conclude that DNA does experience lower diffusivity at a much more restrictive confinement.

Future Work:

For future work, an array of devices will be made, spanning widths from 60-300 nm, and diffusion data will be gathered from all of them. This data will then be used to probe confinement regimes of DNA.

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References:

[1] Levy-Sakin, M. and Ebenstein, Y. (2013). Beyond sequencing: optical mapping of DNA in the age of nanotechnology and nanoscopy. Current Opinion in Biotechnology. 24, 690-698.

[2] Gupta, D., et al. (2014). Mixed confinement regimes during equilibrium confinement spectroscopy of DNA. J. Chem. Phys. 140, 214901-214913.

[3] Lam, E.T., et al. (2012). Genome mapping on nanochannel arrays for structural variation analysis and sequence assembly. Nature Biotechnology. 30(8), 771-776.

[4] Balducci, A., et al. (2006). Double-Stranded DNA Diffusion in Slitlike Nanochannels. Macromolecules. 396273-6281.