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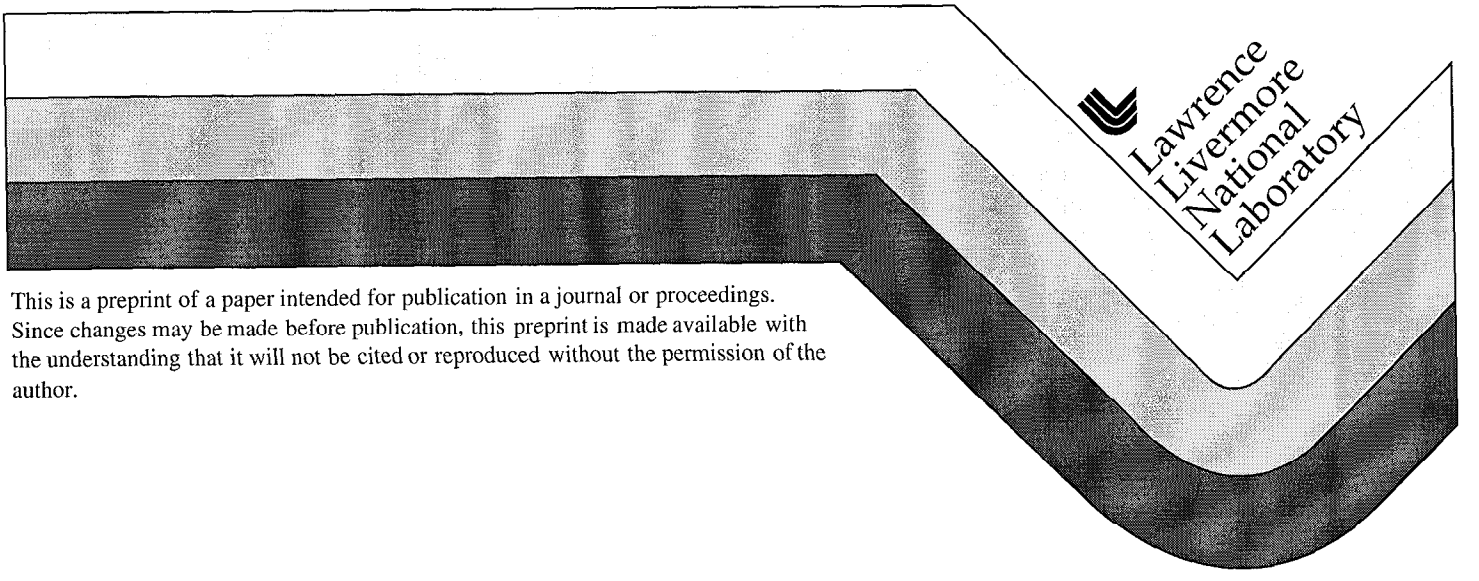

Manipulation of DNA for use in Microfluidic Devices

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MEMS microfluidic systems are becoming increasing popular as a way to integrate sample preparation and biological assays on a single substrate. The resulting reduction in manual operations and reduced reagent use can lead to significant cost savings in performing biological tests. We have explored the use of small scale dielectrophoresis and electrophoresis as a way to manipulate DNA for sample preparation in DNA-based assays. The use of electric fields to manipulate DNA is readily achieved in MEMS devices using standard photolithography techniques to add electrodes to etched flow channels. Dielectrophoresis allows for manipulation of cells and DNA independently of the liquid. This ability is useful in small, valveless fluidic microchips. An advantage of the use of the dielectrophoretic force over an electrophoretic force is that dielectrophoresis works equally well using an AC field, thus reducing trapping of small ions and mitigating electrochemical effects at the electrodes. However, the dielectrophoretic force on the DNA is a function of the volume of the particle; thus, there is a lower practical limit to use of the dielectrophoretic force. Consequently we have also explored methods of manipulating smaller DNA fragments using what we refer to as a stepped electrophoresis method.

For larger-sized DNA fragments, we have explored the limits of dielectrophoretic forces. Dielectrophoresis is a technique wherein a non-uniform electric field is used to induce a dipole in a polarizable particle. This technique is most advantageous as a mechanism for inducing motion in neutral particles, but has been shown to work with charged particles as well. DNA, a negatively charged molecule, exhibits a strong polarization which allows both electrophoretic motion based on its native charge plus dielectrophoretic motion based on induced polarization. In positive dielectrophoresis, the DNA is pulled toward areas of high field strength.

In our studies, we have found that DNA lengths greater than 8.3 Kbases are easily trapped in non-uniform electric fields using interdigitated electrodes with a 30 μm gap in deionized water. Table 1 summarizes results of dielectrophoretic DNA trapping experiments in deionized water as a function of DNA length. Results for experiments performed in Tris-EDTA were similar. For this electrode configuration, we were able to apply 5 V_{rms} to the electrodes before bubble nucleation at the electrodes occurred. Also shown in Table 1 is the dependence of DNA trapping on frequency of the AC source. These results indicate that a range of DNA fragment sizes can be trapped at a frequency of 1 kHz. This characteristic frequency can potentially be used as a method of differentiating DNA from a different biological species. For instance, data collected for *Bacillus Glogii* demonstrates that spores can be captured readily at frequencies much higher than for DNA. This suggests possible fractionation of the various types of biological particles is practical.

Smaller DNA fragments can be concentrated more readily using local electrophoretic motion. Our stepped injection method, depicted in Fig. 1, uses an electrophoresis technique applied over a series of electrodes. A stepped DNA movement is achieved using a set of photolithographically patterned parallel electrodes that can be individually switched between a positive and negative bias. All electrodes are initially held at a positive voltage as a DNA sample is introduced. The electrodes are then sequentially switched negative, repelling the DNA and resulting in net DNA movement. The use of sequential electrodes combined with a stepping mechanism allows us collect DNA over a large area while maintaining low applied voltages (<1.5 V), a desirable feature since higher voltages results in bubble nucleation. The stepped injection method was tested in glass electrophoresis channels, fabricated as described in [1]. The electrodes were 50 μm wide with 30 μm spacing between electrodes. Fig. 2 shows injection of a DNA sample into a water-filled channel using stepped electrophoresis. The benefits of this technique for DNA sample injection include higher signal due to concentration of the DNA sample, improved resolution through compression of the injection plug, and better user-to-user reproducibility for sample injection. This method also does not require any additional space on the electrophoresis "chip", allowing channels to be fabricated parallel to one another for high-density channel arrays.

FIGURES

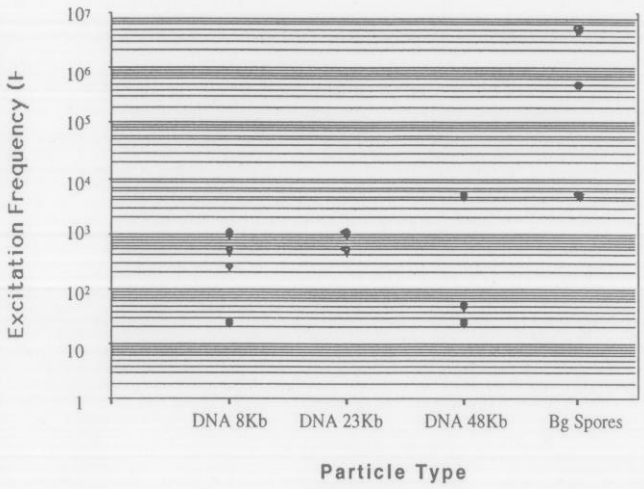

Table 1

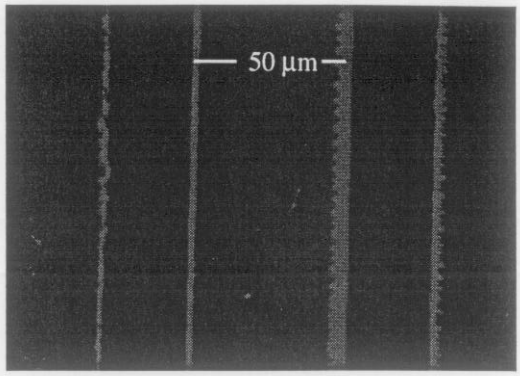

Fig. 1: DNA trapping in the regions of high field strength. DNA is visualized by YOYO dye.

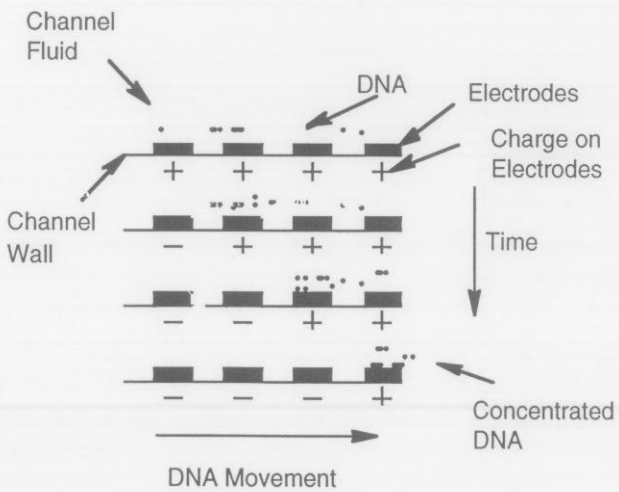

Fig. 2: Stepped DNA injection. Electrodes are sequentially biased to a negative voltage, resulting in net movement of the DNA in the direction of the positively biased electrodes.

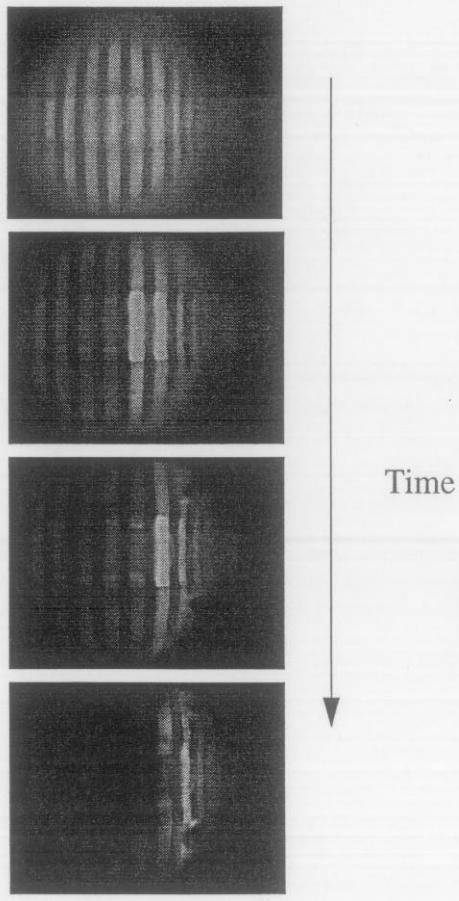

Fig. 3: Top view of the sample input hole to an electrophoresis channel during stepped injection of a 600 bp dsDNA ladder visualized by YOYO dye. DNA was injected into a water-filled electrophoresis channel.

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