LENGTH-BASED SEPARATION OF SHORT DNA USING NANOSLIT ARRAYS
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ABSTRACT
Fused silica nanofluidic devices containing either nanoslit arrays or individual nanoslits were used to investigate the electric field dependent separation of fluorescently labeled, double-stranded deoxyribonucleic acid (DNA) with lengths of 55 bp, 259 bp, and 753 bp. Initial results provided experimental evidence to suggest that the breakdown of Ogston sieving of short DNA can be supplanted at higher average electric fields values in the array, $E_{\text{ave}}$, to recover length-based separation.

KEYWORDS: Nanofluidic, Separation, Electrophoresis, DNA, Nanoslit

INTRODUCTION
Methods for biomolecular separation using nanofluidic devices are of interest for use in integrated and miniaturized analysis systems. For example, nanofluidic arrays consisting of alternating deeper (well) and shallower (nanoslit) regions have been shown to be effective for separating DNA in the kbp range by entropic trapping [1] and DNA below 1 kbp and proteins by Ogston and electrostatic sieving [2, 3]. These studies noted the loss of separation resolution associated with the breakdown of Ogston sieving. Recent theoretical and computer simulation suggest, however, that this breakdown need not represent the upper limit of operation of these devices, and higher $E_{\text{ave}}$ values acting on rod-like DNA in nanoslit arrays with nanoslit depths comparable to the contour length of the DNA may lead to a reversal in elution order and a return to length-dependent separation [4]. To investigate electrophoresis in this high $E_{\text{ave}}$ regime, we fabricated nanoslit arrays with shallower nanoslits and operated these devices at higher $E_{\text{ave}}$ than existing studies [1-3] and examined DNA separation over a range of $E_{\text{ave}}$ values. Our results confirmed a reversal in elution order and renewed separation resolution of short DNA in nanofluidic nanoslit arrays.

EXPERIMENTAL
We performed experiments with fused silica devices fabricated using photolithography, reactive ion etching, and fusion bonding, with the geometries depicted in Figure 1. Figure 2 shows an optical micrograph of an air-filled nanoslit array device. Experiments used 5X tris-borate EDTA buffer containing 2% polyvinylpyrrolidone (v/v) to suppress electro-osmotic flow. DNA samples were 55 bp, 259 bp, and 753 bp and labeled with YOYO-1 fluorescent dye. Constant voltages were applied to electrodes inserted into reservoirs, and electrical current through the separation regions was monitored. Sample plugs containing 55 bp DNA and either 259 bp or 753 bp DNA were launched, and fluorescence was imaged at the end of the separation region for various applied voltages.
Figure 1. Top-down schematics of the two device geometries (nanoslit and nanoslit array) with side-view schematics of the four separation regions.

Figure 2. Optical micrograph showing the intersection and nanoslit array entrance of an air-filled 42 nm deep nanoslit array device (scale bar: 50 μm).

RESULTS AND DISCUSSION

Figure 3 and Figure 4 show representative electropherograms for various \( E_{\text{ave}} \), which was calculated using measured current values, measured buffer bulk conductivity, and the devices modeled as resistors in series. As \( E_{\text{ave}} \) increased, DNA in the nanoslit array devices separated according to Ogston sieving, separation resolution was lost as Ogston sieving failed, and length-based separation returned with the longer DNA eluting first. Because DNA in the nanoslit devices showed no changes in elution order with increasing \( E_{\text{ave}} \), DNA behavior in the nanoslit array devices is attributed to interactions between the DNA and the device geometry.

Figure 3. Electropherograms of 55 bp and 753 bp DNA taken in the 33 nm deep nanoslit device. Elution order did not change for all investigated values of \( E_{\text{ave}} \).
Similar results were obtained for the 745 nm deep nanoslit device and for the 55 bp and 259 bp DNA.

![Graphs showing electropherograms](image)

**Figure 4.** Electropherograms of 55 bp and 753 bp DNA taken in the 21 nm deep nanoslit array device. Elution order changed with increasing $E_{ave}$. Similar results were obtained for the 42 nm deep nanoslit array device and for 55 bp and 259 bp DNA.

**CONCLUSIONS**

The recovery of separation resolution of short DNA at high $E_{ave}$ values using nanoslit array devices would allow for more rapid DNA separation than is possible using Ogston sieving, greatly decreasing the time needed for separation. Additionally, understanding these systems would be an important step towards modeling separations in traditional gel media and realizing on-chip separations of mixtures of DNA, proteins, or other biomolecules with complex shapes.

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**REFERENCES**


