Trapping of DNA in Nonuniform Oscillating Electric Fields

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ABSTRACT DNA molecules can be manipulated in aqueous solution in a manner analogous to optical trapping. Due to the induction of an electric dipole, DNA molecules are pulled by a gradient force to regions of high electric field strength. Molecules can be locally trapped in an oscillating field using strips of very thin gold film to generate strong electric fields with steep gradients. Spatial control over the trapped molecules is achieved because they are confined to a width of ~5 μm along the edges of the gold-film strips. By mixing static and oscillating electric fields, trapped molecules can be moved from one edge to another or made to follow precise trajectories along the edges. This phenomenon should be useful in microdevices for manipulation of small quantities or single molecules of DNA.

INTRODUCTION

DNA molecules in solution carry a negative charge and migrate toward the positive pole when placed in an electric field. In addition to the net charge, the electric field induces a dipole in the molecules. This dipole has few consequences in the quasi-static, homogeneous electric fields that are usually applied for electrophoresis of DNA. However, the induced dipole becomes important when inhomogeneous fields are used, because it renders the molecules sensitive to field gradients. In a diverging electric field, the polarized DNA molecules experience a gradient force that pulls them toward regions of high field strength. The gradient force operates in both static and oscillating electric fields. If the orientation of the electric field is changed, the induced dipole reorients, and the molecules continue to feel a gradient force in the same direction, toward higher field strengths.

A strong dipole can be induced in DNA because each molecule is surrounded by a cloud of counterions that partially neutralize its charge. When an electric field is applied to the solution, the DNA chain and the counterion cloud become distorted, creating a charge separation. This induced polarity is demonstrated by dielectric measurements, which indicate that DNA solutions have a very high dielectric constant in slowly varying (below ~1 kHz) fields (Takashima, 1963; Mandel and Odijk, 1984). Induced dipole effects have also been recognized recently as a source of artifacts in capillary electrophoresis (Mitnik et al., 1995). Upon application of strong electric fields, dipole-dipole attraction causes the DNA molecules to form aggregates, which migrate with anomalous velocity.

Despite these indications that DNA exhibits significant induced-dipole effects, experimental study in this area has been limited. Theoretical work (Ajdari and Prost, 1991) has suggested that trapping by induced-dipole forces could provide an alternative to gel-based sieving methods for size separating DNA, but no practical device was presented in which to test this idea. Washizu et al. (1990, 1994, 1995) constructed the first real devices specifically designed to manipulate DNA by its inducible dipole. In their devices, the DNA fibers were attracted and permanently attached to the electrodes. In an elegant experiment, DNA fibers were stretched and positioned using the effect, and the movement of RNA polymerase was then observed along the fibers (Kabata et al., 1993). Induced-dipole forces (often called dielectrophoresis) have also been used extensively to study and manipulate a variety of larger biological particles such as algae, bacteria, yeast, mammalian blood cells, chloroplasts, and mitochondria (Pohl and Hawk, 1966; Pohl, 1978; Burt et al., 1990; Markx et al., 1994; Marszalek et al., 1995). Optical tweezers, which trap particles in strongly convergent light beams, are a very high frequency form of the same phenomenon (Ashkin, 1992).

Here we report on a practical approach to manipulate DNA molecules by their inducible dipole. Our work differs from that of Washizu and co-workers because the molecules did not become stretched or permanently attached to the electrodes. As a result, we were able to use induced-dipole forces in conjunction with electrophoresis to control movements of the molecules in solution. We believe the method will lead directly to new techniques for the separation and measurement of minute quantities or even individual molecules of DNA.

MATERIALS AND METHODS

Device construction

Standard photolithographic procedures were used to make strips of gold film on coverslip-sized (18 × 18 mm) quartz chips. The strips were fabricated by coating a circular quartz wafer (7.62 cm diameter, 1 mm thick; ESCO Products, Oak Ridge, NJ) with photoresist and then transferring the desired patterns to the photoresist by exposure to UV radiation through a patterning mask. Exposed photoresist was removed to uncover strips of bare quartz. A 2-nm adhesion layer of chrome followed by a 4-nm

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layer of gold was then deposited onto the wafer in a thermal evaporator. Removal of the remaining photoresist left only the strips of metal film that had adhered to the bare quartz. Nine separate patterns were deposited onto a single wafer, which was then diced into 18 × 18 mm squares using a diamond-blade saw. The gold-film strips occupied only the central 10 × 10 mm area of each chip, leaving a 4-mm border of bare quartz surrounding the pattern. A typical pattern with 70-μm-wide strips separated by 30-μm gaps is shown in Fig. 1 *A*.

Video microscopy

The λ phage DNA (Gibco BRL, Gaithersburg, MD) was diluted in deionized water to a concentration of 900 ng/ml. YOYO dye (Molecular Probes, Eugene, OR) was diluted in deionized water to a concentration of 250 nM. Small aliquots of these two solutions were combined 1:4 v/v (DNA: YOYO) just before each experiment, resulting in final concentrations of 180 ng/ml λ DNA (270 nM bp) and 200 nM YOYO. Dilutions were made in deionized water, rather than a buffer, to minimize the conductivity of the samples (see Discussion). Sample conductivities were measured using gold electrodes and a bench top conductivity meter (E-01481–61, Cole-Parmer Instrument Co., Vernon Hills, IL). A 5-ml drop of the fluorescently labeled DNA was sandwiched between a glass coverslip and a quartz chip, causing the fluid sample to spread over the entire 18 × 18 mm area. This arrangement was placed between two platinum wires that had previously been glued onto a regular microscope slide. All four sides of the sandwich were sealed with molten agarose, which also provided the electrical connection between the wires and the DNA sample (Fig. 1 *B*).

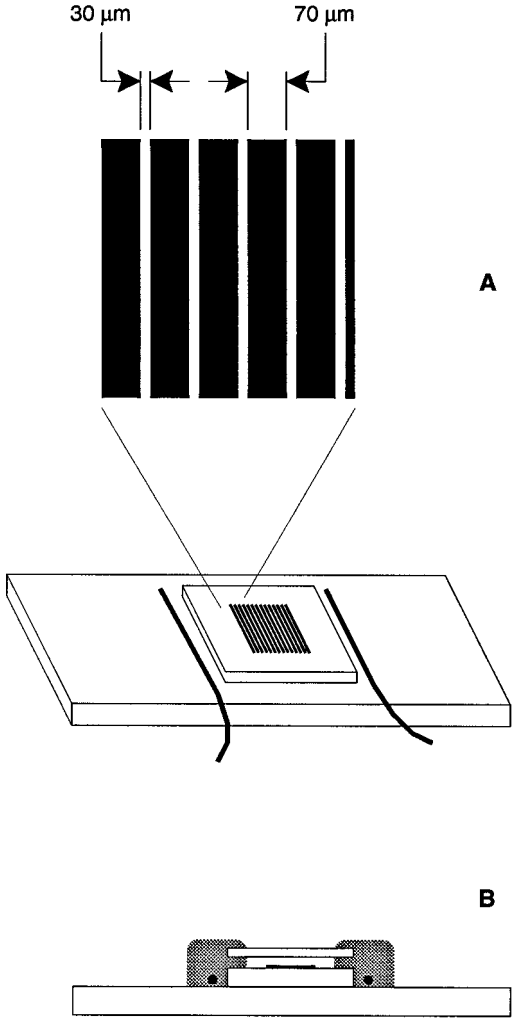

FIGURE 1 Incorporation of strips of thin gold film onto a microscope slide. Standard microlithographic techniques were used to pattern strips of thin gold film onto 18 × 18 mm quartz chips. (*A*) Close-up of a typical pattern with 100 vertical strips of gold film showing the width and spacing of the strips. (*B*) Configuration of the DNA trapping device. A thin layer of stained DNA was sandwiched between the quartz chip and a coverslip. This sandwich arrangement was placed between two platinum wires (shown in dark gray) that had been glued onto a microscope slide. Molten agarose (light gray) sealed the sides of the sandwich and completed the electrical connection between the wires and the DNA sample.

Movement of the DNA molecules was observed in an epifluorescence microscope (Carl Zeiss, Thornwood, NY) and recorded with a sensitive video camera (VE1000SIT, Dage-MTI, Michigan City, IN) and a VCR. Still images were captured from the video tape using-frame grabbing software running on a NeXT workstation. The effects of photo-induced strand breakage were minimized by limiting the duration and intensity of illumination. Many observations were made at low magnification (10× objective lens, NA 0.30). At this low power, individual molecules were not resolved, but fading and strand breakage were significantly reduced. Quick observations could be made at high magnification (60× objective lens, NA 1.25), without causing severe fading or breakage, by shuttering the illumination. When long video sequences (>30 s) were desired, 1% β-mercaptoethanol was included in the DNA sample as an anti-fade agent. The observed behavior of the molecules in all cases was unaffected by the illumination time or by the addition of anti-fade.

Application of electric field

Electric fields were generated in the DNA samples by applying voltage across the platinum wires on either side of the sample. Oscillating voltages were generated by a function generator and could be mixed with steady voltage from a DC power supply before amplification. Typically, a sinusoidal voltage of 200 V peak-to-peak (p-p) was applied across the wires. Although no direct connection was made to the gold-film strips, the electric field in the fluid sample was strongly affected by them. The gold strips acted as short circuits because of their very high conductivity relative to the fluid. Thus, the applied voltage drop was divided among the 99 narrow (30-μm) gaps between adjacent strips, and the large (4-mm) separation between the most peripheral strips and the platinum wires. For a 200-V p-p sinusoid applied to the platinum wires, the voltage drop across each 30-μm gap was only ~0.5 V p-p. Because the gold-film strips were very thin (6 nm), the electric field was highly concentrated near their edges.

The concentration of the electric field by the thin gold-film strips was estimated by solving the Laplace equation using a two-dimensional finite-element model of a cross section through the fluid layer, near the edge of a gold-film strip. Convergence of the model was checked by increasing the number of elements in the model until the results were independent of element density. The calculation indicated that field strengths as high as 190 kV/m, and field gradients of 10 MV/m^2, occurred at the edges of the gold-film strips.

Fluorescence measurements

Measurements of fluorescence were made using a 16-bit cooled CCD camera (TEA/CCD-1317-K/1, Princeton Instruments, Trenton, NJ) attached to an epifluorescence microscope. To record the time behavior of the molecules, the camera was programmed to take a sequence of 250-ms exposures, at a rate of one per second. A computer-controlled shutter (23E-6102A, Ludl Electronic Products, Hawthorne, NY) allowed the molecules to be illuminated only during CCD exposures. This significantly reduced bleaching and breaking of the molecules, eliminating the need for anti-fade (>60% of the initial fluorescence remained after 90 exposures). The first exposure was taken before the application of voltage. The oscil-

lating voltage was switched on just before the second exposure and switched off again after 30 s had elapsed. Because the DNA was distributed uniformly during the first exposure, this image was used for background subtraction. Profiles of fluorescence across the gold-film strips were obtained from the digital images by integrating the images parallel to the strips. The profiles were normalized to account for the slow decrease of fluorescence over the course of each experiment.

RESULTS

Fig. 1 shows a simple device that reversibly traps DNA molecules using induced-dipole forces. To trap DNA, the confining force must overcome thermal forces that tend to disperse the molecules randomly. DNA trapping by the gradient force requires strong, highly divergent, oscillating electric fields. These conditions were met by placing strips of very thin (6-nm) gold film in contact with the DNA solution. Typically a 30-Hz, 200-V p-p sinusoid was applied across the solution, generating an oscillating electric field in the fluid. The strips have much higher conductivity than the surrounding medium, which combines with their extreme thinness to concentrate the electric field at their edge. We estimate that field strengths of at least 190 kV/m occurred at the edges of the gold-film strips.

Before application of voltage, individual molecules were scattered throughout the sample (Fig. 2, *A* and *C*). Upon application of the oscillating field, the DNA was drawn toward the edges of the gold strips and trapped there at high concentration. Molecules that happened to be near ($<10\ \mu$m from) an edge when the field was turned on were immediately trapped. Molecules that began farther away took longer to become trapped, but thermal motion and weak convection currents brought nearly all of the molecules into the traps within ~1 min (Fig. 2, *B* and *D*). The molecules did not become stuck to the gold. While trapped, the molecules were free to move along the edges of the gold strips.

Trapped molecules wiggled perpendicularly to the edge of the gold strips, with the frequency of the applied voltage. This wiggling is visible in Fig. 2 *D*, where the motion gives the molecules a streaked appearance. We attribute this be-

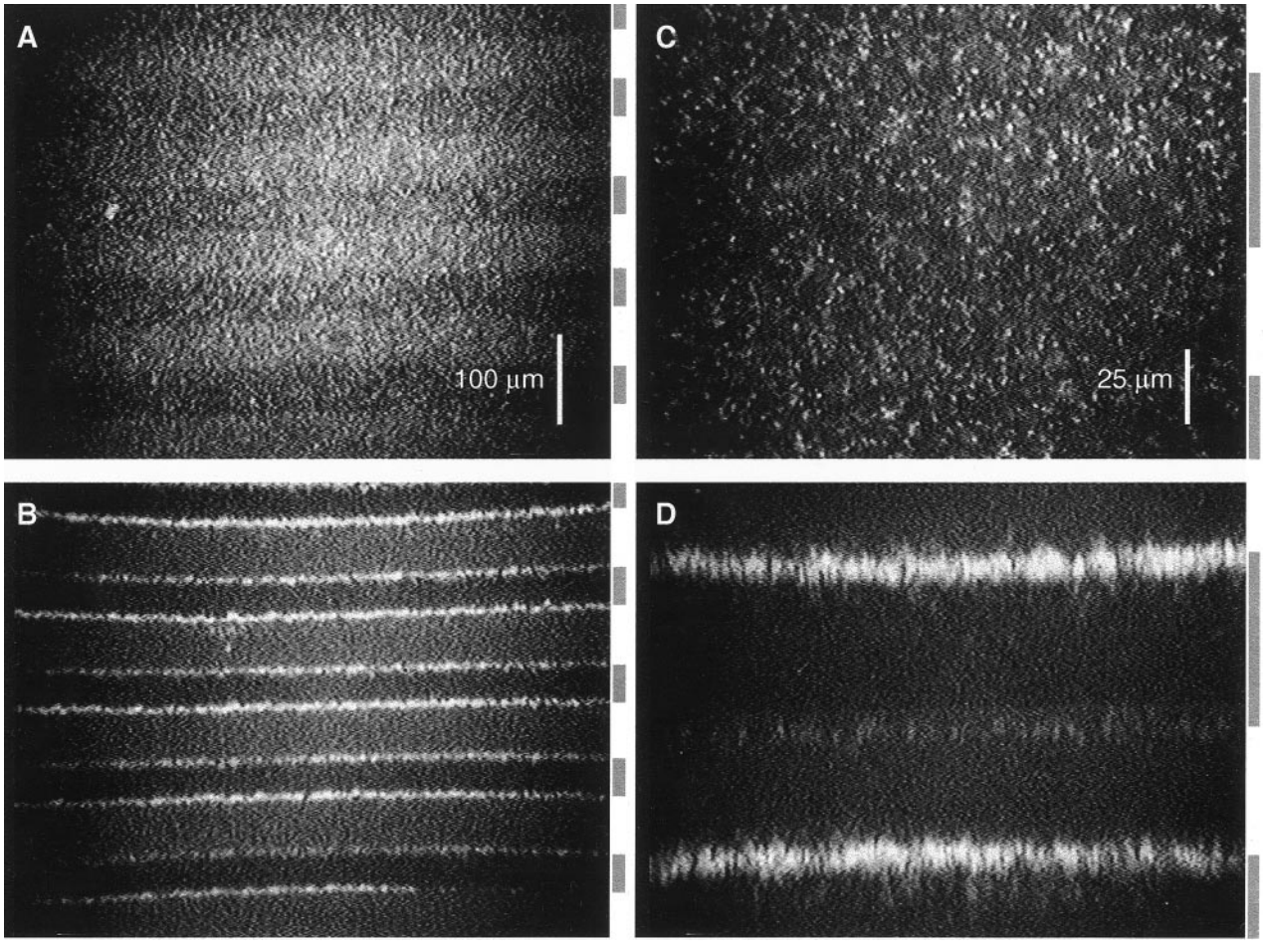

FIGURE 2 Trapping of stained DNA molecules over the edges of the gold-film strips. (*A* and *C*) Before applying the electric field, individual YOYO-stained λ DNA molecules were scattered randomly throughout the sample. (*B* and *D*) 1 min after applying a 30-Hz, 200-V p-p signal to the platinum wires, nearly all of the molecules became trapped over the edges of the films. Gray bars on right indicate the positions of the strips of gold film in the figures. The streaked appearance of the trapped molecules in *D* occurs because they were wiggling with the frequency of the applied field.

havior to electrophoretic motion caused by the net charge of the DNA. Electroosmotic motion of the fluid may also contribute to this behavior.

The voltage required for DNA trapping increased with frequency in the range of 1 Hz to 10 kHz. At low frequencies (1–10 Hz) trapping could occur at voltages as low as 30 V p-p applied to the platinum wires. This applied voltage corresponds to a voltage drop of only ~80 mV p-p across the gap between two adjacent gold-film strips. Trapping at 1 kHz required 200 V p-p across the platinum wires. The 30-Hz, 200-V p-p sinusoid used most often in our experiments trapped λ DNA molecules (48.5 kb) rapidly and effectively.

We quantitated the fluorescence of trapped DNA molecules by taking a continuous sequence of CCD exposures while the voltage was turned on and then off again. By integrating the digital images parallel to the gold-film strips, brightness profiles perpendicular to the strips were obtained. When the trapping field was switched on, narrow peaks of fluorescence (~5 μm in width) grew rapidly in the profiles, where the DNA molecules became concentrated over the edges of the strips (Fig. 3, *A–C*). When the field was switched off again, the peaks broadened and shortened as the DNA molecules dispersed (Fig. 3, *D* and *E*).

The time behavior is illustrated in Fig. 4, where the average height of the 15 peaks in Fig. 3 is plotted against the elapsed time. The growth of the peaks upon application of the trapping field is well fit by a single-exponential function, $A(1 - e^{-t/\tau}) + 1$, with amplitude $A = 2.8$ and time constant $\tau = 5.4$ s. The spreading of the DNA after the field is turned off is governed by diffusion, and therefore the broadening and shortening of the peaks is expected to follow a multi-exponential function (the higher spatial modes decaying more quickly than lower ones). In practice, a double-exponential function, $Be^{-t/\beta} + De^{-t/\delta} + 1$, with $B = 2.2$, $\beta = 5.0$ s, $D = 1.4$, and $\delta = 47$ s, provides an adequate fit. The data in Figs. 3 and 4 are typical for the trapping of λ DNA, but it should be noted that the repeatability of this experiment was poor. Rise-time constants, τ, obtained for identical samples measured in the same devices, varied between 2.6 and 18 s. Rise amplitudes, *A*, varied between 0.49 and 4.1.

When purely oscillating fields are applied to the DNA sample, there is no net charge transfer from electrode to solution. Relatively high field strengths can thus be produced even in solutions that contain salts without inducing bubble formation or chemical reactions at the edges of the gold strips. However, the DNA-trapping effect is sensitive to the ionic composition of the solution. We have observed DNA trapping in buffer solutions typically used in gel electrophoresis (TBE: 89 mM Tris/borate, pH 8.3, 2 mM Na_2·EDTA) and in the presence of a variety of other molecules (proteins and lipids, data not shown). We have also achieved DNA trapping from whole bacterial lysates (*Escherichia coli* containing a high-copy-number plasmid, lysed by resuspension in hypotonic solution, data not shown).

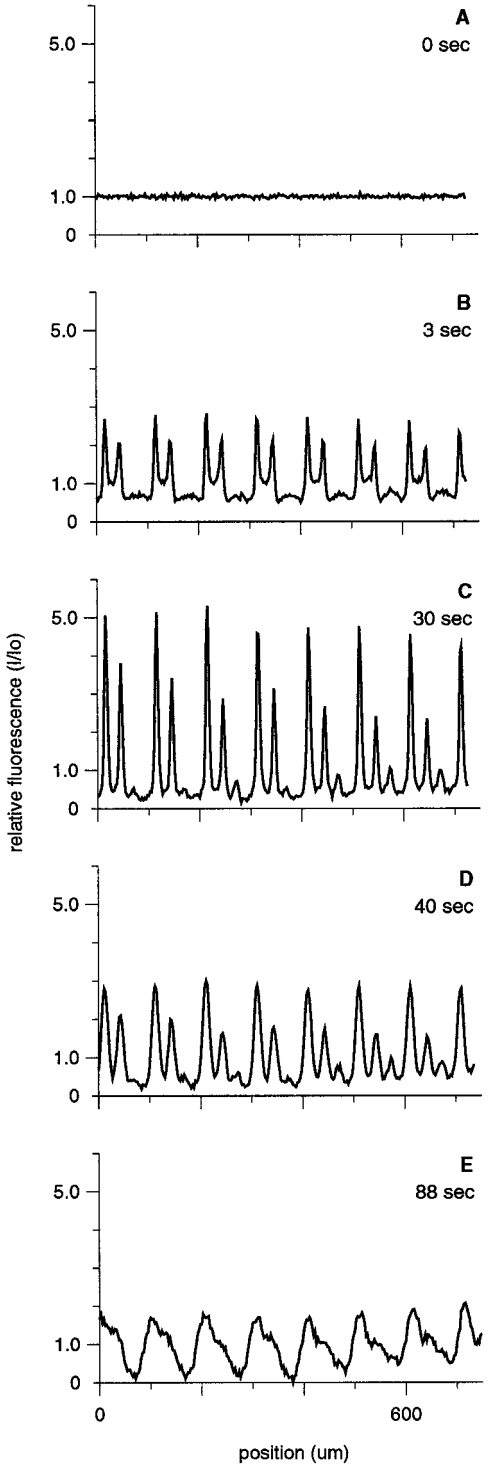

FIGURE 3 Profiles of fluorescence from trapped DNA molecules. Fluorescence profiles were obtained from CCD exposures by integrating the digital images parallel to the edges of the gold-film strips. Selected profiles from a sequence taken as the trapping field was switched on and then off again show the build-up and dispersion of trapped DNA molecules. (*A–C*) Immediately after the trapping field was switched on, narrow peaks of fluorescence grew rapidly in the profiles, where the DNA molecules became concentrated over the edges of the strips. (*D* and *E*) After 30 s, the field was switched off again, and the peaks broadened and shortened as the DNA molecules dispersed. The asymmetry in the peaks was caused by a slight DC offset (≤1 mV) that was difficult to eliminate from the oscillating voltage applied across the devices.

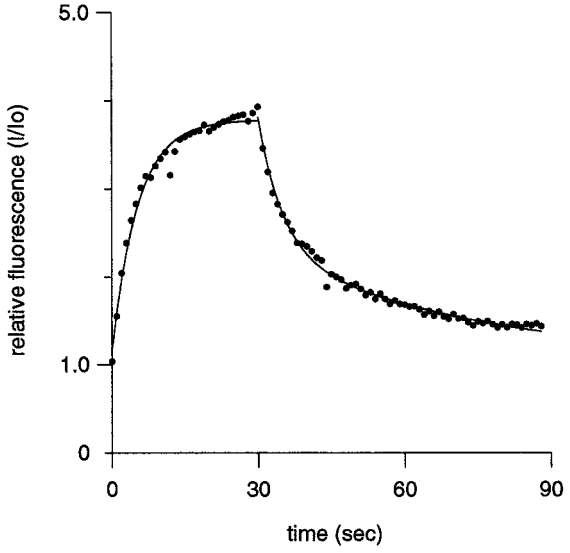

FIGURE 4 Time course of DNA trapping. The average height of the 15 peaks from the sequence of Fig. 3 is plotted against the elapsed time. The growth of the peaks upon application of the trapping field is fit by a single-exponential rise, $A(1 - e^{-t/\tau}) + 1$ with amplitude $A = 2.8$, and time constant $\tau = 5.4$ s. A double-exponential function, $Be^{-t/\beta} + De^{-t/\delta} + 1$, with $B = 2.2$, $\beta = 5.0$ s, $D = 1.4$, and $\delta = 47$ s, adequately fits the shortening of the peaks that occurred after the field was turned off.

Trapping was not effective in 1% SDS solution or isotonic saline.

By mixing a short pulse of constant voltage with the oscillating trapping voltage, it was possible to control movement of the molecules perpendicular or parallel to the edges of the gold-film strips. A short pulse of sufficient strength (5 V across the platinum wires, or ~12 mV per gap) applied perpendicular to the strips could dislodge molecules from one trap and transport them just far enough so that they settled into the next trap (Fig. 5). Trapped molecules could also be made to move along the edges of the strips. To produce this type of motion, the periodic trapping voltage (30 Hz, 200 V p-p) was applied perpendicular to the gold strips while a slight constant voltage (5 V) was simultaneously applied parallel to the strips. Under these conditions, the DNA molecules remained in the traps but moved slowly along them (data not shown).

The behavior of the DNA molecules in our experiments was usually quite uniform over several contiguous fields of view. However, variation was often observed over an entire slide in the number of molecules trapped per unit length of edge or in the width of the zone occupied by trapped molecules. In the experiments combining constant and oscillating fields to move molecules, it was possible to control movement in one area of the slide, but uniform movement everywhere on the slide was rare. These variations in behavior appeared to correspond to differences in the thickness of the fluid layer, which was not well controlled in our experiments. In areas with a thinner fluid layer, fewer molecules were trapped per unit length of edge, and the trapped molecules were confined to a narrower zone. Such

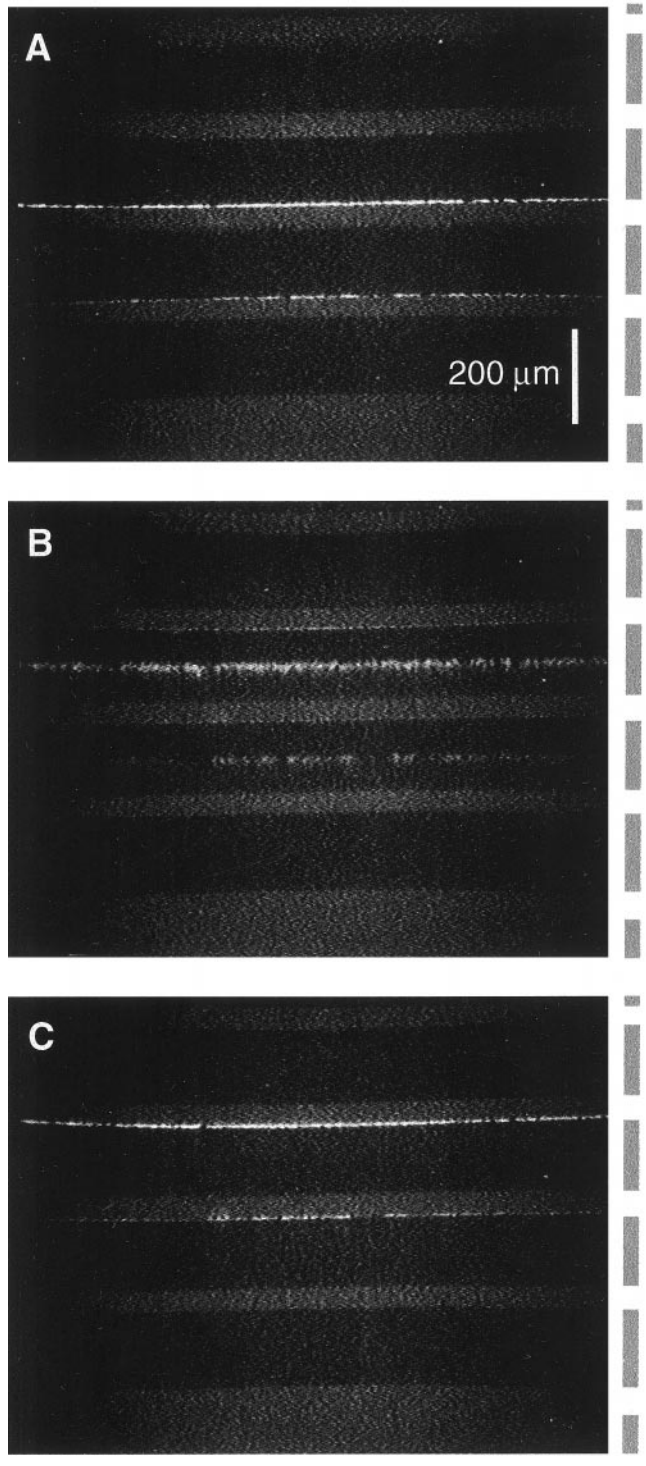

FIGURE 5 Controlled movement of DNA molecules from one trap to another. (*A*) Applying a purely oscillating field traps molecules over an edge. (*B*) Mixing a pulse of constant voltage with the oscillating trapping voltage dislodges molecules from the trap, moving them upwards in the image. (*C*) The pulse duration is chosen so that molecules travel just far enough to settle into the next trap. Gray bars on right indicate the positions of the strips of gold film in the figures. Molecules could also be made to move along the traps by applying the oscillating trapping voltage perpendicular to the gold-film strips while a slight steady voltage was simultaneously applied parallel to the strips (data not shown).

variation is not surprising because the fluid is part of the electrical circuit. Variations in the fluid thickness affect the local electrical resistance of the solution and therefore the local electric field.

DISCUSSION

Motion of DNA in an electric field is not only affected by the net charge carried by the molecules but also by the dipole induced in the molecules by the field. The induced dipole results in a gradient force, which pulls the molecules toward regions of high field strength. In our devices, this gradient force was exploited to trap the molecules in the high-field-strength regions that occurred near the edges of thin strips of gold film. Use of purely oscillating fields was required for efficient dipole trapping of DNA because the molecules were both charged and polarized and therefore experienced a superposition of the charge and dipole effects. In purely oscillating fields, the effect of charge was minimized, merely causing the molecules to wiggle in place, and the DNA could be manipulated by its induced dipole alone.

Trapping of DNA could occur over a range of frequencies (1 Hz to 1 kHz), but trapping at the higher frequencies required more applied voltage. A likely explanation for the lower trapping efficiency at higher frequencies is that the dipoles induced in the DNA become weaker as the frequency is increased. Indeed, dielectric measurements have shown a steep decrease in the polarizability of DNA molecules in solution as the frequency increases in this range (Takashima, 1963; Mandel and Odijk, 1984).

The sensitivity of the trapping effect to salt concentration in the solution is a common feature of systems that use induced-dipole forces (Burt et al., 1990; Markx et al., 1994). The generation of a strong induced dipole depends on a mismatch between the polarizability of the particle and that of the surrounding medium (Wang et al., 1992). If the conductivity of the solution is too high, then the difference between the particle and the medium polarizabilities can be slight, resulting in a weak induced dipole (Takashima, 1967). This may explain why we could observe trapping in TBE buffer (which had relatively low conductivity, 630 μS/cm) but not in isotonic saline (14 mS/cm), or 1% SDS (1.2 mS/cm). The samples of DNA diluted and stained in deionized water had very low conductivity (1.8 μS/cm).

Slight differences in the conductivity of the samples may also explain why the values of rise-time constant t and amplitude A obtained from the CCD image sequences were not repeatable. With a sample volume of only 5 μl, very small amounts of contaminating salt can significantly alter the conductivity. Variations in the geometry of the fluid layer may also have been a major factor contributing to the variation. With better control of these two factors, we expect to make repeatable quantitative measurements that will allow comparison of different samples.

The gradient forces generated in our devices created potential energy wells around the edges of the gold-film strips. We do not know the exact shape of these wells, but a conservative estimate for the well depth can be made by assuming a one-dimensional, V-shaped well that occupies 5 μm of the total width (50 μm) of the periodic structure. The depth necessary to trap a given fraction of the molecules is then determined by integration of the Boltzmann distribution inside and outside the wells. Our observations indicate that at least 99% of the molecules are being trapped, which gives a depth (at the tip of the V) of $\sim 9\ k_B T$ (Boltzman constant). (If the well is extremely narrow, then its depth would need to be somewhat more than this. For example, a 0.5-μm well width requires a depth of $\sim 12\ k_B T$ to trap 99% of the particles. A width less than 0.5 μm is unreasonable because it would be narrower than the DNA molecules, which have a radius of gyration of $\sim$0.5 μm.) Using the gap spacing of 30 μm as a characteristic length, this means that the DNA molecules were subjected to gradient forces in the femtonewton range.

The possibility of using dipole trapping to size separate DNA molecules was predicted on purely theoretical grounds by Ajdari and Prost (1991). Their model predicts an order of magnitude improvement in size selectivity and speed over conventional gel-based sieving methods, but these predictions were not tested in a real experiment. The first real experiments using induced-dipole forces to manipulate DNA were conducted by Washizu and co-workers (1990, 1994, 1995). The authors reported that application of an extremely strong ($>$2 MV/m), very-high-frequency (MHz) electric field to a DNA solution caused the molecules to stretch out to their full (double-helix) length ($\sim$17 μm in the case of λ DNA). The molecules elongated in the direction of the electric field and then were drawn toward the edges of the aluminum films used to apply the field (Washizu and Kurosawa, 1990). When the molecules reached the aluminum, they became permanently attached at one end to the film. Molecules immobilized in this way could then be used as enzymatic substrates, allowing many novel applications (Kabata et al., 1993; Washizu et al., 1995).

In contrast to these previous experiments, DNA trapping in our experiments was reversible. The molecules did not become elongated or permanently stuck to the gold-film strips, and they could diffuse freely within the traps. The differences may be due to our use of inert metals for the thin-film strips and weaker, lower-frequency electric fields than Washizu et al. Additionally, we were able to control movements of the DNA in solution. These capabilities can form the basis of a miniature system for manipulation of DNA and lead to a variety of useful devices.

For example, a DNA trap placed at the entry of a capillary electrophoresis (CE) channel could greatly improve the resolution of this technique. Very fast size separations of DNA fragments have been achieved by performing CE in tiny arrays of microfabricated channels (Woolley and Mathies, 1994). As with conventional CE, the devices must be filled with a sieving buffer (such as hydroxyethylcellulose), and the resolution of the separation is limited by the

width of the initial injection plug of DNA (300–500 μm in the microchannels). A DNA trap could concentrate the molecules into a 5-μm injection plug, improving the resolution 100-fold.

DNA traps could also be used as the basis for a size separation device that would not require a sieving buffer, as proposed by Ajdari and Prost (1991). In such a device, the transit time of DNA molecules through a series of traps would vary according to differences in the dipole-trapping efficiency of the fragments.

Another successful approach to rapid DNA sizing has been fluorescent burst measurements by flow cytometry (Goodwin et al., 1993; Huang et al., 1996). The fluorescence signals from single DNA molecules are extremely weak, but they could be enhanced if the transit time of the DNA molecules in the laser spot was lengthened. If instead of hydrodynamic focusing, a DNA trap was used to control the trajectory of the molecules as they traversed the light spot, then the transit time could be increased 100-fold (from 1 to 2 ms) resulting in proportionally larger fluorescence signals and better resolution.

DNA trapping may find uses in microdevices that integrate several steps of a molecular biology experiment into a single device. Already many techniques, including gel electrophoresis, polymerase chain reaction, and hybridization reactions are being performed in very low volumes (Huang et al., 1992; Chen and Dovichi, 1994; Woolley and Mathies, 1994, 1995; Meldrum et al., 1995; Rayner et al., 1995; Shalon et al., 1996; Woolley et al., 1996). Miniaturization can save time and reagents in each of these individual techniques, but loading and recovery of the liquid volumes present difficulties. Therefore, the biggest improvements will come when several techniques can be integrated onto a single chip. Dipole traps could be used to hold DNA molecules in place as other reagents are washed out (e.g., contaminating debris) or washed in (e.g., enzymes) or to direct small amounts of sample to specific locations within a device (e.g., an area for hybridization). With the current trend toward miniaturization in molecular biology, manipulation of minute quantities of DNA by electrical forces is emerging as an important technology. Because strong, highly divergent electric fields are easily generated in miniature devices, an understanding of induced-dipole effects is essential.

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