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# Nonlinear Electrophoretic Velocity of DNA in Slitlike Confinement

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## ABSTRACT

We have applied zero-time-averaged alternating electric fields to DNA molecules in a cross-shaped nanofluidic slit. We observed a net drift of DNA molecules, the magnitude of which depends on the square of the electric field amplitude. From the rate of accumulation of DNA at the center of the device, we derive an estimate for the second order electrophoretic mobility,  $\mu_2$ . We observe that focusing is absent at a dipole rotation frequency  $>20$  Hz, which suggests that  $\mu_2$  depends on the frequency of the alternating fields. The observation of a nonzero  $\mu_2$  raises the possibility of frequency dependent electrophoretic DNA separation by length achievable in the absence of a sieving matrix.

## INTRODUCTION

The theory of polyelectrolyte electrophoresis has a long and complicated history [1–3]. Particularly for highly charged semiflexible biopolymers, like double-stranded DNA, there is still considerable uncertainty about which approximations are appropriate in finding an analytical expression for the electrophoretic mobility [4]. The complications arise from several interrelated factors, discussed in some detail by Hoagland *et al.* [5].

To briefly summarize, the origin of one of the complications is the so-called diffuse counter-ion cloud that surrounds the polyelectrolyte out to the Debye length, which depends inversely on the square root of the ion concentration [6]. In this discussion we consider a solution with added salt as in our experiment. In a salty solution, the Coulomb interaction between point charges is exponentially screened by the Debye length in the limit of the linearized Poisson-Boltzmann equation for small surface potentials [7]. Under the application of an external electric field, the counter-ions are driven with a force equal in magnitude to the electric force on the polyelectrolyte and opposite in direction. The force transmitted to the polyelectrolyte through the viscous medium is less though since the hydrodynamic interactions are screened over a similar scale [1]. Another more important complication, in the context of our experiment, results when the symmetry of the diffuse counter-ion cloud is deformed due to the motion of the polyelectrolyte with respect to the solvent. This effect has traditionally been termed ‘relaxation’, or ‘asymmetry field’, and results in a slowing of the polyelectrolyte due effectively to the dipole distribution of the ion cloud that opposes the applied field [8]. The force transmitted to the polyelectrolyte through the solvent by the motion of the ion cloud previously discussed must be corrected due to the distortions in the cloud. Consequently, there is a complicated dependence on the polyelectrolyte mobility with the ionic concentration. For long DNA molecules in a monovalent salt solution, it has been observed experimentally that the mobility decreases monotonically with the ionic strength [5].

The importance of several of these complications are lessened when one is able to linearize the Poisson-Boltzmann equation for small surface potentials. This approximation does not appear valid on its face for double-stranded DNA with two negative charges every base pair (0.34 nm) when in an aqueous solution with neutral pH. Manning's theory of counterion condensation [9], however, posits that the charge on a polyelectrolyte in a salt solution is renormalized by the counterions when the spacing between charges is less than the Bjerrum length. The Bjerrum length equates the Coulomb energy of two unit charges in solution to the thermal energy. For DNA, this indicates that its bare charge should be reduced by about 75% from its nominal value [10] making the Debye-Hückel approximation more reasonable. However, while helpful in explaining some observed aspects of polyelectrolyte dynamics [11], the counterion condensation theory has been criticized for containing unphysical assumptions [4].

Electrophoresis of DNA within a confining fluidic structure, like a nanoslit, adds more complications to the story. The confining structure modulates the conformation and dynamics of the DNA in ways that have been extensively studied in experiment, theory, and simulation [12–15]. The most relevant aspects for our experiment result from the extension of the DNA molecule's equilibrium size and the increase in its longest relaxation time with the confining slit height ( $h$ ). According to the de Gennes blob model in moderate slit confinement, the equilibrium size and the longest relaxation time scale as  $h^{-1/4}$  and  $h^{-7/6}$  respectively [16]. Additionally, since the confining slit walls are negatively charged in aqueous solution in our experiment, the ionic double layer that extends approximately a Debye length from the walls can be important. This double layer can overlap at low salt concentration or small slit heights, though we are far from that realm in our experiment. More importantly, the applied external electric field can drive bulk fluid flow through electroosmosis due to the convection of the positive salt ions in the double layer near the walls (in the direction opposite to that of the electrophoretic force on the DNA) [6].

To summarize, the most relevant issues of polyelectrolyte electrophoresis for our experiment results from the relaxation of the diffuse ions surrounding a DNA molecule as well as the DNA relaxation time being a function of the confining slit height. It should further be noted that theories of electrophoresis do not consider changes to the equilibrium conformation of the polyelectrolyte at moderate electric field strengths. However, Tang *et al.* [17] showed that coupling of electrohydrodynamic flows can cause compression of individual DNA molecules in bulk under constant applied electric fields.

## THEORY

We investigate the possibility that the DNA electrophoretic velocity ( $v$ ) depends non-linearly on the electric field ( $\vec{E}$ ). Experiments such as those done by Campbell [18] indicate that, though the electrophoretic velocity is commonly assumed to be proportional to the electric field in free solution, statistically significant deviations from linearity are observed for  $\lambda$  DNA confined in nanocapillaries with dimensions in the 100 – 500 nm range. We therefore make the assumption that

$$\vec{v} = (\mu_1 + \mu_2 E) \vec{E} \quad (1)$$

where  $E$  is the magnitude of  $\vec{E}$ , and  $\mu_1$  is the commonly referred to electrophoretic mobility. Concentration of DNA exploiting this nonlinear dependence of electrophoretic velocity on electric field has previously been demonstrated in an agarose gel [19,20]. Following a similar method, we apply an electric field that consists of the superposition of a dipole field ( $\vec{E}_d$ ) rotating at angular frequency  $\omega$  and a quadrupole field ( $\vec{E}_q$ ) rotating at  $2\omega$ . The dipole field is given by

$$\vec{E}_D = E_{0D}[\cos(\omega t)\hat{x} + \sin(\omega t)\hat{y}]. \quad (2)$$

The coordinate system is shown overlaid on a schematic of the central region of the cross-shaped slit in Fig. 1. The magnitude  $E_{0D}$  is  $E_{0D} = \Delta V/L$ , where  $L$  is the distance separating two electrodes. The quadrupole field is given by

$$\vec{E}_Q = -E_{0Q} \cos(2\omega t)[x\hat{x} - y\hat{y}], \quad (3)$$

where  $x$  and  $y$  are the horizontal and vertical distance from the origin respectively. The magnitude  $E_{0Q}$  is complicated by the geometry of the device used in the experiment. In our device,  $E_{0Q}$  is approximately equal to  $(E_{0D}/80 \mu m)$  within the central region (Fig. 1), as further detailed in the Discussion section. Substituting  $\vec{E} = \vec{E}_D + \vec{E}_Q$  into Eq. (1) and taking the time average, we see that the term proportional to  $\mu_1$  will not contribute. The term proportional to  $\mu_2$ , however, is significantly more complicated. It can be shown [34] that the time averaged velocity in the limit that  $E_{0D} \gg E_{0Q}$  is given by

$$\langle \vec{v} \rangle = -\frac{1}{4}\mu_2 E_{0D} E_{0Q} \vec{r}, \quad (4)$$

where  $\vec{r}$  is the radial vector in two dimensions. We refer to this configuration of fields as *focusing* because DNA molecules at nonzero  $\vec{r}$  will on average drift radially toward the center.

If we change the initial phase of the quadrupole field such that

$$\vec{E}_Q = E_{0Q} \cos(2\omega t)[x\hat{x} - y\hat{y}] \quad (5)$$

then the time-averaged velocity in the limit that  $E_{0D} \gg E_{0Q}$  is given by

$$\langle \vec{v} \rangle = \frac{1}{4}\mu_2 E_{0D} E_{0Q} \vec{r}. \quad (6)$$

We refer to this mode as *defocusing* because molecules not at the center should drift radially outward from the center on average. The results for the time-averaged drift velocity in Eqs. (4) and (6) were confirmed by numerical integration of Eq. (1) in the described limit.

The focusing and defocusing behavior were qualitatively confirmed in a simple computer simulation written in python. The simulation employed a Runge-Kutta method to update the trajectory of a point particle subject to electrophoretic drift from the combined dipole and quadrupole field, and thermal diffusion for a lambda DNA molecule in an approximately 250 nm deep slit based on experimental results [16].

We note that if we assume the velocity has a non-linear dependence on the electric field as shown in Eq. (1), that the time averaged velocity for either the dipole field alone ( $\vec{E} = \vec{E}_D$ ) or the quadrupole field alone ( $\vec{E} = \vec{E}_Q$ ) is zero. This result is straightforward for  $\vec{E} = \vec{E}_D$ . When  $\vec{E} = \vec{E}_Q$ , the result depends on the time average of  $|\cos(2\omega t)| \cos(2\omega t)$  being zero.

## EXPERIMENTAL SECTION

Devices were fabricated on 500 micron thick double-sided polished fused silica wafers (Mark Optics) using standard contact photolithographic techniques. The nanoslits were reactive ion etched using a CHF<sub>3</sub>-O<sub>2</sub> plasma (Oxford Instruments), then measured using a Dektak-150 profilometer. A nanofluidic device consisted of four perpendicular legs that were each 0.75 cm long, 36 microns wide, and approximately 253 nm deep. The legs were oriented perpendicular to each other such that they formed a cross. The interior corners of their intersection, which we refer to as the central region, were rounded

hyperbolically to avoid edge effect artifacts in the applied electric fields, the equation of the hyperbola being  $y = 1000 \mu\text{m}^2/x$ . The hyperbolic curve of the walls extended in the domain  $20 \mu\text{m} < |x| < 50 \mu\text{m}$ . At distances greater than  $50 \mu\text{m}$  from the center,  $y$  was constant and the legs had a constant width. Each leg terminated in an access hole, made via sandblasting. Here a reservoir for fluid and a gold electrode could be inserted. We refer to the four reservoirs for a given device as left, right, top, and bottom. The etched wafer was cleaned and touch bonded to a fused silica cover wafer (160 microns thick, Mark Optics), then slowly heated to  $1050^\circ\text{C}$  in a Thermo Scientific Lindberg/Blue M box furnace to make the bond permanent.

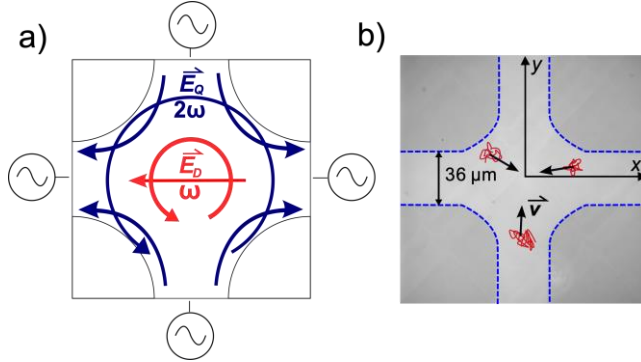


Figure 1. a) Schematic of the rotating quadrupole (blue) and the rotating dipole (red) electric fields that are summed to create the *focusing* field orientation within the central region of the cross-nanoslit device. The four legs extend  $0.75 \text{ cm}$  and terminate in fluid reservoirs where electrodes are inserted in order to apply the fields (not shown). b) An optical micrograph of the central region at  $40\times$  magnification taken in brightfield with the walls of the device traced (blue-dashed line). A cartoon of DNA molecules is overlaid (red), showing the direction of the drift velocity of each molecule (radially inward) during the application of the focusing field.

Fluid reservoirs were formed by gluing pipette tips, with the ends removed, using 732 silicone rubber sealant (Dow Corning) over the access holes. The buffer consisted of  $445 \text{ mM}$  Tris-borate and  $10 \text{ mM}$  EDTA ( $5\times$  TBE), with  $3\%$  by volume  $\beta$ -mercaptoethanol added to reduce photobleaching. The ionic strength of this buffer is estimated to be about  $160 \text{ mM}$ .

$\lambda$  DNA ( $48.5 \text{ kbp}$ , New England Biolabs) was prepared at a concentration of  $1.6 \times 10^{-10} \text{ M}$  and stained with YOYO-1 dye (Thermo Fisher Scientific) at a ratio of  $5:1 \text{ bp:dye}$  molecules. In a typical focusing experiment,  $3 \mu\text{L}$  of stained  $\lambda$  DNA was added to  $57 \mu\text{L}$  of  $5\times$ -TBE in the left reservoir. In a typical defocusing experiment,  $10 \mu\text{L}$  of stained  $\lambda$  DNA solution was added to  $50 \mu\text{L}$  of buffer in the left reservoir in order to increase the ambient concentration of DNA in the central region. DNA was loaded into the channel overnight with a  $45 \text{ V/cm}$  electric field applied between the left and right reservoirs.

The electrode potentials were applied using a National Instruments PCI-6229 DAQ card controlled by LabView, and amplified by four LTC2057HV op amps (Linear Technology), chosen for their small input offset voltage and low thermal offset output drift. Electrical contact was made with the buffer in the reservoirs using gold electrodes.

A dipole field is created by applying a potential difference between opposite legs (left-right or top-bottom). A quadrupole field is created by applying a symmetric potential difference (e.g.  $+30 \text{ V}$  and  $-30 \text{ V}$ ) between perpendicular legs (left-top, right-top, left-bottom, and right-bottom), and zero potential difference between opposite legs (left-right or top-bottom). The

dipole and quadrupole fields are shown schematically in Fig. 1a. In both focusing ( $\vec{E} = \vec{E}_D + \vec{E}_Q$ ) and defocusing ( $\vec{E} = \vec{E}_D - \vec{E}_Q$ ) configurations we used a value of  $3\pi$  rad/s for  $\omega$  (the dipole was rotated at a frequency of 1.5 Hz, and the quadrupole at 3 Hz). The instantaneous sum of the quadrupole and dipole potentials was rounded to one of four discretization values for convenience. Each discrete potential was held for a length of time equal to  $1/12^{\text{th}}$  of a dipole period ( $T$ ) as shown in Fig. 2a.  $T/12$  was chosen as the time interval for discretization arbitrarily. The discrete voltage values for the focusing orientation were +15 V, 0 V, -15 V, and -30 V. For the defocusing orientation the values were +28 V, +14 V, 0 V, and -14 V. The values are not symmetric because the superposed field is also asymmetric in time as shown in Fig. 2.

To eliminate capacitive coupling leading to small biases in the drift of the DNA, 24 American Wire Gauge shielded tray cable (Automation Direct) was used to connect the DAQ to the printed circuit board (PCB) where the amplifiers were mounted. Additionally, a ground plane was added between traces on the front and back of the PCB, and signal traces were kept a minimum of 1 cm apart. The circuit board was designed in Autodesk Eagle and fabricated by OshPark.

The fluorescent DNA was observed using a Evolve EMCCD camera (Photometrics) connected to an Olympus IX71 inverting microscope using a 40x, 0.75 numerical aperture objective (Olympus). An X-Cite Series 120PC mercury lamp was used to excite the YOYO dye. Images were taken with an exposure time of 140 ms using a custom LabView program approximately every 15 minutes and processed using custom software written in Matlab.

Between experiments, the buffer was removed from the reservoirs, replaced with DI water, and the reservoirs were covered with parafilm to prevent evaporation. Intermittent blockages of the entrance to the nanoslit in the reservoir region were cleared with dilute sulfuric acid (1.8 M) if we were unsuccessful in loading DNA after several trials. Afterwards, the devices were rinsed repeatedly with 5X-TBE and allowed to equilibrate for at least 24 hours before the next experiment.

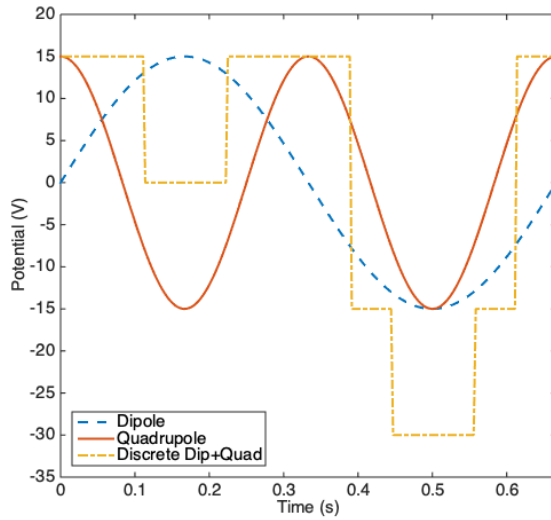


Figure 2. The potential pattern applied to one electrode in the focusing configuration. The mathematical quadrupole (red, solid) and dipole (blue, dashed) potentials are summed and discretized to four possible outputs (+15 V, 0 V, -15 V, or -30 V) as represented by the square wave (yellow, dot-dashed). Despite being asymmetric in time, the discretized dipole plus quadrupole potential integrates to zero over one dipole period.

## RESULTS

We experimentally observed DNA entering the central region during the application of the focusing field configuration with  $\omega = 3\pi$  rad/s (1.5 Hz dipole frequency) over the course of 22 hours. The amount of DNA in the central region increased linearly over time after approximately one hour as shown in Fig. 3. Images were taken roughly every 15 minutes for 5 hours. The fields were applied for an additional 17 hours without the 15 minute interval images being taken, after which the final images were taken as shown in Fig. 3f.

In order to establish that the focusing effect was caused by the superposition of the dipole and quadrupole fields rather than either in isolation, experiments were conducted with  $\vec{E} = \vec{E}_Q$  and separately with  $\vec{E} = \vec{E}_D$ , with  $\omega = 3\pi$  rad/s in each case. A negligible amount of DNA was seen to enter the central region during the application of each as seen in Fig. 4. Occasionally, a DNA molecule was observed to stick to the surface of the glass. These were removed by applying a 45 V/cm field from left to right for about 15 s. Images containing stuck DNA were not used in the analysis.

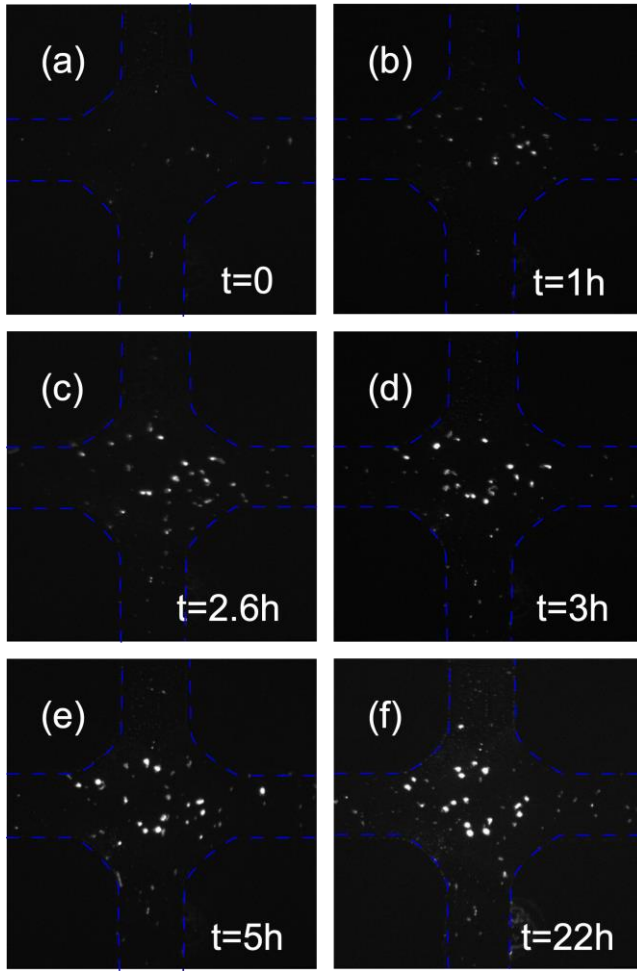


Figure 3. (a) Fluorescent image of the central region of the cross-slit showing  $\lambda$  DNA at the start of the application of the focusing field orientation rotating at a dipole frequency of 1.5 Hz. Walls are traced (blue-dashed line). Magnification is 40x. Fluorescent image of the central region after applying the focusing field for one hour (b); 2.6 h (c); 3 h (d); 5 h (e); 22 h (f). Although fragments of  $\lambda$  DNA are evident, a clear increase in the fluorescent intensity, or DNA concentration, is observed over time.

The rate of accumulation of DNA in the focusing configuration was determined using a custom Matlab algorithm. In order to select for regions containing fluorescently labelled  $\lambda$  DNA, each frame was binarized so that pixels below an intensity threshold were revalued at zero, while those above the threshold were revalued at one. The threshold intensity was determined by averaging Otsu's method [21] over several images from near the middle of the focusing experiment. In addition, bright objects below an area of 5 connected pixels were removed to eliminate camera noise. We refer to the number of pixels which pass these criteria as  $N_+$ . We refer to the total number of pixels in an image as  $N$ . We then plotted the ratio  $N_+/N$  versus the time from the start of the experiment as shown in Fig. 4. The data was fit to a linear function by a least squares estimate. Only data from images after the first DNA molecule passing the previously discussed criteria was seen to enter the central region ( $\sim 1$  hr) was used in calculating the best-fit line. The uncertainty was determined from a binomial distribution algorithm for the observed probability of  $N_+$  bright pixels appearing in a sequence of  $N$  binary pixels. The slope of the best fit line in the focusing experiment was  $(5.39 \pm 0.17) \times 10^{-4}$  / hour where the uncertainty represents the standard error. The slope of the best fit line in the defocusing experiment was  $(-1.92 \pm 0.20) \times 10^{-3}$  / hour.

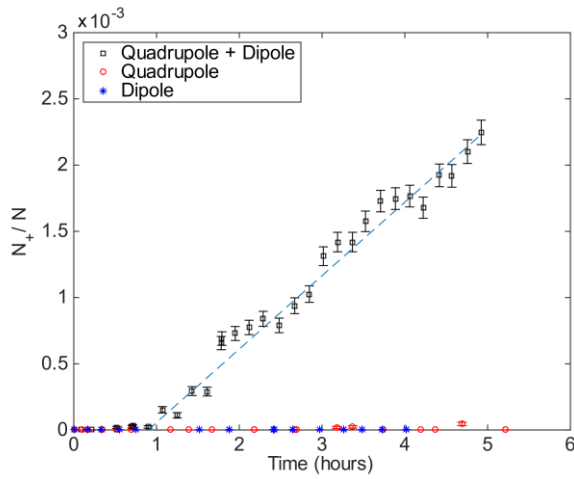


Figure 4. Plot of the fraction of connected pixels passing a brightness threshold in images of the central region over time for the focusing (quadrupole and dipole) field (black squares), quadrupole only (red circle), and dipole only (blue star) fields. The data are fit to a linear function by a least squares estimate (blue-dashed line).



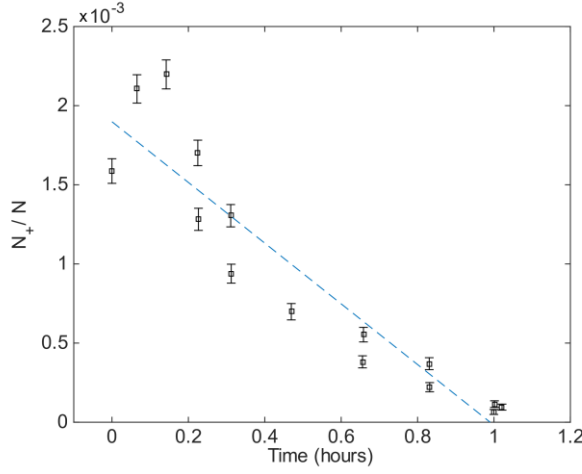


Figure 5. Plot of the fraction of connected pixels passing a brightness threshold in images of the central region over time for the defocusing (dipole and inverted quadrupole) field. The data are fit to a linear function by a least squares estimate (blue-dashed line).

## DISCUSSION

DNA was seen to accumulate in the central region during the application of the focusing orientation of fields as seen in Fig. 4a. The rate ( $R$ ) at which DNA molecules were entering the central region during the application of the focusing fields was determined from the slope of the best linear fit ( $\Delta N_+/\Delta t$ ) as follows:

$$R = \frac{1}{A} \frac{\Delta N_+}{\Delta t}, \quad (7)$$

where  $A$  is the average area of a DNA molecule in the central region. From the rate, we extracted an estimate for  $\mu_2$  using the following expression derived from Eq. (4)

$$\mu_2 \approx \frac{2 R L}{E_{0D} E_{0Q} r'}, \quad (8)$$

where  $L$  is the average distance between DNA molecules projected onto the x-y plane (parallel to the confining slit walls), representing the average inter-particle distance before the application of the focusing fields, and  $r$  is the radial distance from the origin to the border of the central region. The factor of 2 in the numerator results from DNA being able to enter the central region from either the left or right.

The average area of a molecule of  $\lambda$  DNA in the central region was found to be  $42 \pm 19$  pixels, corresponding to approximately  $6 \mu\text{m}^2$ . No attempt was made to correct for the point spread function of the optical system, as we are only seeking an order of magnitude estimate. Using  $L$  inferred from the loading concentration of the DNA and the cross-sectional area of the channel, we estimate  $\mu_2 = 1.5 \times 10^{-6} \text{ cm}^3/(\text{V}^2 \cdot \text{s})$ . The separation of molecules along the loading axis was observed to be about 7% larger than the calculated value. We note that this value of  $\mu_2$  implies that the speed of DNA would be about double its known value in a DC field [22] at  $\sim 270 \text{ V/cm}$ . The somewhat higher estimate for  $\mu_2$  than we expected adds further credence to our conclusion that the emergence of a nonlinearity in  $v$  may be frequency dependent, since it has not previously been observed in similar experiments in bulk solution or in a confined geometry with DC fields.

Additionally, the DNA concentration in the central region was observed to decrease with time during the application of the defocusing field as seen in Fig. 5. Accordingly, we determined an estimate for  $\mu_2$  based on the best fit ( $\Delta N_+/\Delta t$ ) for the rate of DNA exit from the images taken during the application of the defocusing field using the following expression:

$$\mu_2 \approx \frac{-R L}{E_{0D} E_{0Q} r} \quad (9)$$

The factor of two, relative to Eq. (8) results from the DNA being able to exit the central region through all four legs. This expression gives an estimate of  $\mu_2 = 1.7 \times 10^{-6} \text{ cm}^3/(\text{V}^2 \cdot \text{s})$ . We believe that the larger spread in the data at the start of the application of the defocusing fields is an artifact of the loading concentration of the DNA being larger than in the focusing experiment. The higher loading concentration was chosen to ensure that a significant amount of DNA would be present within the central region before the application of the fields.

The first order mobility ( $\mu_1$ ) of  $\lambda$  DNA was also measured within our cross nanoslit device. Videos were taken of several molecules moving electrophoretically under a constant electric field with magnitude ranging between  $10 - 30 \text{ V/cm}$ . The velocities were determined via custom software particle tracking in Matlab. The first order mobility was found to be  $(2.41 \pm 0.15) \times 10^{-5} \text{ cm}^2/\text{V} \cdot \text{s}$ . This value differs significantly from the mobility value for long DNA in bulk [22]. The first order mobility at approximately this degree of confinement has previously [18,23] been measured to be about  $9 \times 10^{-5} \text{ cm}^2/\text{V} \cdot \text{s}$ . The difference is likely due to an increase in electroosmotic flow in our experiment. We conducted an additional experiment at lower salt concentration using 1x-TBE as the buffer. The DNA molecules were then observed to move toward the lower potential electrode in the loading field, leading us to conclude that the electroosmotic flow was larger than the electrophoretic force at this salt concentration. Other researchers have added polyvinylpyrrolidone (PVP) to the buffer in nanochannel devices to suppress the electroosmotic flow [23,24]. However, it was demonstrated that the PVP can act like a sieving matrix [25], introducing an additional source of nonlinearity in the electrophoretic velocity of DNA molecules. Previous experiments also indicate that the observed mobility can vary significantly from day-to-day [26,27].

There are several approximations in our analysis. We used a finite difference method numerical solution to the Poisson equation in two dimensions to evaluate the dipole and quadrupole fields separately. The dipole field is not uniform in the entire central region. The necessity of having a limited width to height ratio in a nanofluidic slit to prevent collapse when bonding introduces a limitation that would not be present were the dipole field to fill a full 2D square region. The numerical model indicates that the magnitude of the dipole field in the legs perpendicular to the dipole direction decays exponentially with a decay length of approximately 80 microns. This decay in the off-axis dipole magnitude puts a limit on the distance from which DNA can be effectively focused in the cross nanoslit.

The numerical model of the quadrupole field indicates that it is zero at the origin and linearly rises to approximately the same magnitude as the on-axis dipole field within about two channel widths ( $80 \mu\text{m}$ ) of the origin, where it remains constant. It is known that the quadrupole field is extensional in the central region [28] (i.e., the field strength along a given axis is proportional to distance along that axis). The electric field lines in the central region were qualitatively verified to approximate a quadrupole by following tracer DNA moving under a constant applied quadrupole field. As discussed in the Supporting Information [34], Eq. (4) for the drift velocity is only exact when the magnitude of the quadrupole ( $E_{0Q}$ ) is much smaller than the magnitude of the dipole ( $E_{0D}$ ), which is approximately true within the central region.

It is possible that the physical properties of the buffer could change as it is heated by the electrical current passing through it in such a way as to make  $v$  appear nonlinear with  $E$ , as is the case in high field experiments in agarose [29]. We calculate a projected rise in temperature ( $T$ ) per time ( $t$ ) due to Joule heating in our experiment of

$$\frac{\Delta T}{t} = \frac{F V_{rms}^2}{R C m} \approx 3 \times 10^{-3} \text{ } ^\circ\text{C/h} \quad (10)$$

where  $V_{rms}$  is the root mean square voltage,  $C$  is the specific heat of water ( $4,184 \text{ J/kg} \cdot \text{K}$ ),  $R$  is the resistance of the nanoslit (measured to be  $\approx 4000 \text{ M}\Omega$ ),  $m$  is the mass of buffer in the nanoslits and reservoirs, and  $F$  is a unit conversion factor. It then seems quite unlikely that Joule heating plays a role in the inception of  $\mu_2$  over the 5 hour interval in our experiment. We also ignore the possible complicated effect of intermolecular DNA interactions within the central region, which may need to be included in future measurements of higher precision. We note that the maximum concentration of DNA achieved in the central region still appears to be lower than the crossover into the semi-dilute regime where inter polymer entangling has traditionally been considered to start [30], and therefore we consider it unlikely to play a role.

We also investigated the frequency dependency of  $\mu_2$  in a limited range due to experimental constraints. Though Eq. (4) did not predict a dependence of  $\mu_2$  on the dipole frequency, the 1.5 Hz dipole frequency at which the experiments were performed was chosen as it is slightly higher than the frequency at which focusing was observed in more viscous agarose by Marziali, *et al.* [20] As shown in Fig. 1 in the supporting information [33], the focusing effect in our device was not observed at a dipole frequency  $>20 \text{ Hz}$  over a 6 hour period. We surmise that the relaxation time of the DNA, approximately 0.5 s for lambda DNA in a 250 nm deep slit [16], may play a role in the frequency dependence of  $\mu_2$ . Additionally, the extended conformation of the DNA in the confining slit may affect the relaxation time of the surrounding diffuse ion cloud as discussed previously. We therefore consider it likely that there is a resonant frequency, effected by the depth of the confining slit and DNA contour length, at which DNA focusing is most effective. The lower limit of the explorable frequency range is set by the width of the central region. At too low a frequency, the molecule is carried a significant distance outside the central region, where the approximation of  $E_{0D} \gg E_{0Q}$  no longer holds, and the focusing drift speed is not well characterized. The width of the central region could likely be slightly increased in the future without causing the collapse of the channels.

The drift velocity within the central region was found to be too small to be well characterized when tracking a single molecule due to the time limit set by eventual photobleaching of the molecule. Single molecule observations should be possible in the future in a device with a wider central region, or increased electric field strength across the central region by using shorter legs or larger voltage amplification.

Investigation as to whether the field dependence in  $\mu$  is an emerging electrokinetic effect like that described by Bazant *et al.* [31,32] could be done by performing similar focusing experiments with increased field strength in the center (either by altering the nanofluidic device or using more powerful electronics), and extracting an estimate of  $\mu_2$  as we have done. If the emergence of  $\mu_2$  is induced by high AC fields, it is likely that the value of  $\mu_2$  will vary with the maximum field magnitude.

In the future, we intend to explore the degree of confinement dependence of  $\mu_2$ , up to measurements in free solution, to establish the slit-depth for several DNA contour lengths that optimizes the focusing effect. The frequency dependence of  $\mu_2$  requires further theoretical and experimental investigation. We would like to improve the resolution of our measurement by utilizing a stronger electric field to reduce the timescale of the experiments. This can be accomplished by reducing the length of the perpendicular legs connecting the central region to the reservoirs where the electrodes are inserted and by using higher

voltage amplifiers. With moderate design improvements, we envision that the device could be used to selectively concentrate DNA within a narrow range of contour lengths in the central region, by utilizing the appropriate focusing frequency, for subsequent analysis either on or off chip.

## CONCLUSION

We have performed experiments demonstrating focusing and defocusing of  $\lambda$  DNA in nanoslits. Additionally, we extracted a first order estimate of the nonlinear mobility term ( $\mu_2$ ) of the electrophoretic velocity ( $v$ ) with the electric field strength ( $\vec{E}$ ). Since we do not observe a focusing effect for a dipole rotation frequency of  $> 20$  Hz, we infer that there is a  $\mu_2$  dependence on frequency that requires further investigation. We are fabricating new devices to measure  $\mu_2$  more accurately at various dipole rotation frequencies in the 1-100 Hz range. In the future, we intend to explore the confinement dependence of  $\mu_2$  for single DNA molecules within nanoslit devices and in free solution over a range of increased electric field magnitudes. We also intend to expand the size of the central region of our devices to facilitate single molecule study of focusing DNA.

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- [34] See Supplemental Material at [URL will be inserted by publisher] for the derivation of the drift velocity expression.