Tilted post arrays for separating long DNA

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Recent simulations by Chen and Dorfman [Electrophoresis **35**, 405–411 (2014)] suggested that “tilting” the electric field with respect to the lattice vectors of a hexagonal post array would lead to a substantial improvement in electrophoretic DNA separations therein. We constructed such an array where the electric field is applied at an angle equidistant between the two lattice vectors. This tilted array leads to (i) baseline resolution of 20 kbp DNA and λ DNA (48.5 kbp) in a 4 mm channel and (ii) measurable separation resolutions for electric fields up to 50 V/cm, both of which are improvements over untilted post arrays of the same post density. The predicted time required to reach a resolution of unity is approximately 5 min, independent of electric field. The separations are more reproducible at higher fields. © 2014 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4884521>]

I. INTRODUCTION

Hexagonal post arrays are one microfluidic method for rapidly separating long DNA, typically tens to hundreds of kilobase pairs (kbp).^{1,2} Agarose gel electrophoresis can only separate these long fragments using pulsed electric fields.³ Unfortunately, due to the slow migration of long DNA in the net direction of the applied field, pulsed field separations take hours to days to achieve a baseline separation;³ this time is compared to minutes needed to separate long DNA in post arrays.^{4–6} Regular arrays of micro- and nano-sized posts, fabricated by semiconductor methods, have been the subject of substantial experimental work^{6–19} using a wide variety of materials, post sizes, and post spacing. In addition to microfabricated arrays with perfectly ordered features, results also exist for self-assembled magnetic beads,^{4,5,20} which form quasi-hexagonal arrays, microfabricated arrays with intentional disorder,²¹ and nanowire-based posts.^{22,23} Given the extensive experimental work on DNA electrophoresis in post arrays, let alone the corresponding efforts using computer simulation,¹ it would seem that further work in this area would lead to merely incremental advances in the device performance. In the present contribution, we argue otherwise by exploiting the orientation of the electric field with respect to the lattice vectors of the array,²⁴ i.e., DNA electrophoresis in a “tilted” post array. This previously underutilized design parameter leads to a substantial improvement in separation resolution and time compared to conventional hexagonal post arrays without introducing any complicated nanofabrication methods.^{6,10}

Our interest in the orientation of the electric field with respect to the lattice vectors characterizing the two dimensional, crystalline array arises from the deleterious effect of “channeling” during DNA electrophoresis in a post array, a concept first identified in two-dimensional Brownian dynamics simulations by Patel and Shaqfeh.²⁵ Figure 1 illustrates the basic idea behind the channeling effect. When the DNA disengages from a collision with the post, its center-of-mass is slightly to the side of the post. If the electric field is applied along the low porosity lattice vector (Fig. 1(a)) or the high porosity lattice vector (Fig. 1(c)) of a hexagonal array, which are the standard approaches in experiments, the DNA will travel through these channels at its free solution mobility until it collides with another post. When the electric field strength

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FIG. 1. Schematic illustration of an electric field applied at (a) 0° tilt, (b) 15° tilt, and (c) 30° tilt. The lines illustrate the direction of the applied voltage drop. Only tilt angles between 0° and 30° need to be examined due to hexagonal symmetry, and we have arbitrarily assigned the 0° tilt as the lattice vector corresponding to the low porosity direction.

is high, which is desirable for fast separations, the DNA can spend the majority of their time inside the channels. The mobility of the DNA in the array thus approaches the free solution mobility, which is independent of molecular weight, and the separation is lost.

The channeling effect was explored more deeply by Mohan and Doyle,^{26,27} using three-dimensional Brownian dynamics simulations, and their simulation results suggested that post arrays with regularly spaced patterns, such as a hexagonal array, would fail to separate long DNA due to the channeling effect. This conclusion was at odds with experimental work demonstrating separations in such arrays,⁶ and the disagreement between simulation and experiment was ultimately resolved by recognizing that the curved field lines in electrically (and ionically) insulating post arrays break up the channeling effect to some extent.¹⁵ The channeling effect described in simulations only dominates when the array is so sparse that the electric field lines are hardly perturbed by the posts.¹⁶ However, since transport across field lines occurs by diffusion, the effects of channeling seem to limit separations in sparse arrays to electric fields in the range of 10–20 V/cm.^{4,5,17} At higher electric fields, the time to diffuse onto a “collision” field line is long compared to convection through the array¹⁶ and the separation fails.

Further improvement in DNA separations in post arrays thus requires a way to remove the channeling effect. One approach to break up the channels is tilting the array, as in Fig. 1(b), such that the macroscopic voltage drop is no longer coincident with the lattice vectors of the array. Brownian dynamics simulations²⁴ indicate that tilted arrays should preserve the cyclic rope-over-pulley mechanism^{8,28,29} underlying the separation without producing the dispersive, multi-post collisions that can occur at higher post densities. Indeed, these simulations²⁴ indicate that tilting the post array increases the collision probability while leaving the hold-up time unchanged, so the speed difference between different sized DNA molecules increases upon tilting the array without greatly affecting the dispersion.²⁴ The model used for these simulations has been well parameterized with post array experiments and has been shown to match single molecule experiments in normal hexagonal post arrays very well.¹⁸

To date, Brownian dynamics simulations²⁴ are the only evidence supporting the efficacy of tilted post arrays compared to their untilted counterparts. In the present contribution, we test these simulation results by experiments in a post array with a 15° tilt, as illustrated in Fig. 1(b). Our results confirm that a tilted post array results in an improved separation when compared to the standard hexagonal post array, but we do observe some differences between the optimistic predictions of the Brownian dynamics simulations and our experimental results.

II. MATERIALS AND METHODS

We created the tilted post array, consisting of $1\ \mu\text{m}$ diameter posts with a $3\ \mu\text{m}$ center-to-center spacing, using projection photolithography on a 4 in. silicon dioxide coated silicon wafer. The oxide layer was grown by placing the silicon wafer in a 1000°C furnace (Tylan) with an oxygen and hydrogen rich environment (36% oxygen, 64% hydrogen) for 1 h. This resulted in a silicon dioxide layer of about 370 nm, as measured by ellipsometry. SPR 995 CM 0.7 photoresist (Rohm & Haas) was spun on the wafer at 3000 rpm for 30 s then exposed on a Cannon i-line projection lithography system (Cannon FPA 2500 i3) at $155\ \text{mJ}/\text{cm}^2$ with a $-0.2\ \mu\text{m}$ focus

offset. Using the photoresist as an etch mask, the silicon dioxide was etched away using reactive ion etching (Surface Technology Systems 320). The exposed silicon was then etched to a depth of about $4\ \mu\text{m}$, with the oxide layer serving as the etch mask, using a deep reactive ion etching system (Plasma-Therm SLR 770). Through holes were etched for the reservoirs using a KOH wet etch at 90°C , using the photoresist PSB (ProTEK) as the etch mask. The device was then oxidized for 4 h at 1100°C in an oxygen rich environment to provide electrical insulation. Finally, the device was sealed with a borosilicate wafer using an anodic bonding process (Karl Suss SB6) and then diced using a wafer saw (Disco), resulting in the final working device in Fig. 2.

For the electrophoresis experiments, the device was initially filled with ethanol for easy wetting. After initial wetting, the device was soaked overnight in a 1% (w/v) solution of polyvinylpyrrolidone (Sigma, 40 kDa) in water. Finally, the device was mounted onto the chip holder and filled with a $5 \times$ TBE buffer (Sigma) at a pH of 8.1 with 0.07% (w/v) polyvinylpyrrolidone and ascorbic acid (Sigma) and 3% (v/v) β -mercaptoethanol (Sigma). The holder was then placed on an inverted microscope (Leica DMI-4000). Platinum electrodes, inserted into each reservoir, were connected to a high voltage power supply (Labsmith HVS-1500) to control and monitor the potentials in the four reservoirs. Fluorescence intensity data were collected using a photomultiplier tube (Hamamatsu H7422-04), which collected the data through a $40\times$ objective situated at a finish line located 4 mm from the start of the post array. The data were collected at a rate of 1 kHz. To test the reproducibility of the separation, the experiments were performed over 5 separate days using two different devices; the experiments for days 1 and 2 were performed in one device (chip 1) and the experiment for days 3, 4, and 5 were performed in another device (chip 2).

The DNA used in the experiments was a mixture of λ DNA (New England Biolabs), which is 48.5 kbp, and 20 kbp DNA (Thermo Scientific). The DNA was dyed with YOYO-1 intercalating dye (Life Technologies) at a concentration of 1 dye molecule per 5 bp by staining for 24 h. The dyed solution was added to the loading reservoir ((b) in Fig. 2). A small plug of DNA was injected into the separation channel using a standard shifted-T injection scheme.³⁰ If the device was to be used again the next day, the DNA solution in reservoir B was replaced with buffer and the device and holder were wrapped in parafilm so the reservoirs would not dry out. If the device was not going to be used the next day, it was soaked first in 30% hydrogen peroxide to clean out any residue and then the hydrogen peroxide solution was replaced with a 1% solution of polyvinylpyrrolidone (Sigma) in water for storage.

III. RESULTS

The natural metric for assessing the quality of the separation is the resolution³¹

$$R = \frac{t_\lambda - t_{20}}{2(\sigma_\lambda + \sigma_{20})}, \quad (1)$$

where for a finish line separation, t_i is the average time for species i to cross the finish line and σ_i^2 is the variance of the peak of species i . To obtain these quantities, we used the sum of two

FIG. 2. A schematic of the overall device and a scanning electron micrograph of the 15° tilted post array separation matrix. The letters in the reservoirs indicate the labeling convention used later. The 4 mm long separation matrix is in the region indicated by the red, dashed circle.

FIG. 3. Representative electropherograms for 10 V/cm, 30 V/cm, and 50 V/cm for 20 kbp and 48.5 kbp DNA with finish line detection at 4 mm inside the array. The peak identities are labeled only for the 30 V/cm electropherogram. The inset presents the resolution as a function of electric field obtained over multiple devices and multiple days.

Gaussian functions. The main panel of Figure 3 shows representative electropherograms obtained in the 15° tilted post array at 10 V/cm, 30 V/cm, and 50 V/cm after 4 mm of separation distance. In all cases, the two peaks are easily resolved; the resolution exceeds 0.7 at 50 V/cm in about 3 min, is approximately 1 for 30 V/cm after 5 min, and more than 1.5 at 10 V/cm in 15 min. The inset of Fig. 3 shows the resolution data as a function of electric field obtained over multiple devices and multiple days for electric fields between 10 V/cm and 50 V/cm in 10 V/cm intervals.

In addition to the separation resolution, reproducibility of the separation is also a figure of merit. Figure 4 provides a scatter plot of the resolution as a function of the time t_λ for the slower moving peak to reach the detector. The plot contains the data for all of the experiments performed in the tilted array. Interestingly, we found that the separations performed at the higher electric fields led to more reproducible separation resolutions and elution times. To investigate the reproducibility in more detail, Fig. 5 plots the average velocity and width of the elution peak as a function of electric field, where the error bars correspond to the standard deviation of the quantity over the set of experiments ($n = 6-12$). As we can see, much of the variability of the resolution is attributable to the variability in the peak width, with the uncertainty in the velocity being much smaller and relatively constant as a function of electric field. From

FIG. 4. Scatter plot of the resolution and time required for the slower λ -DNA peak to reach the detector. The data were collected over 5 days and in two different devices. The red data are from chip 1 and the blue data are from chip 2.

FIG. 5. Average velocity and the variance of the peak width for the 20 kbp DNA and 48.5 kbp DNA as a function of electric field strength. The error bars correspond to the standard deviation of the data set. The dashed lines are included to guide the eye.

an analytical chemistry perspective, the key variable for reproducibility is the ratio of the mobility (or, equivalently, the ratio of the elution times) for the two species, provided that the resolution is sufficient to measure this quantity. The standard deviation of the mobility ratio, relative to the mean mobility ratio, is 3% at 10 V/cm and 30 V/cm, 2% at 20 V/cm, and 1% at 40 V/cm and 50 V/cm. Thus, it would be relatively easy to size the DNA by using one of the molecular weights as a standard. The reproducibility of the mobility ratio, even in the presence of fluctuations in the elution time, is consistent with previous experiments in post arrays.¹⁶

We also performed experiments up to 80 V/cm in the devices, but we were not able to fit the peaks for electric fields exceeding 50 V/cm. At electric fields around 130 V/cm, the oxide layer suffers electrical break down,³² so our upper bound of 80 V/cm is a conservative estimate to avoid destroying the device.

IV. DISCUSSION

A. Comparison with other post array separations

The separation resolutions and times in Fig. 3 compare favorably with prior results using the same post diameter and center-to-center spacing, but the 0° tilt orientation in Fig. 1(a).¹⁷ Explicitly, the latter experiments¹⁷ used a 12.5 mm separation channel to obtain a separation resolution of 1.22 after 20 min at 10 V/cm. Indeed, our results compare favorably to all of the post array separations we could identify that used a similar range of DNA to our 20 kbp and 48.5 kbp mixture. The most closely related separations in the literature are the first experiments performed in a magnetic bead array,⁴ which required 800 s to get a resolution of 1.0 when separating 33.5 kbp and 48.5 kbp. Note that subsequent experiments in the magnetic bead array system⁵ improved on the overall separation quality but did not revisit these particular molecular weights.

It is useful to consider how the tilted array compares with other strategies for removing the channeling effect. One idea is to make a random arrangement of posts to break up the ordering due to the channels^{25,26} while maintaining a pattern that can still be produced using typical microfabrication.²¹ If the array is sufficiently dilute, then this strategy can increase the frequency of collisions compared to an ordered array of the same density.²⁶ Unfortunately, once the array density increases to that used for successful separations in micropost arrays (e.g., the post size and center-to-center distance used in our experiments), the DNA will often engage with multiple posts in the denser regions of the system, leading to increased band broadening²¹ due to the wide variance in holdup times for such long-lived collisions.^{33–35} As a result, it is not surprising that there are no experimental data available for separations in micropatterned, disordered arrays.

A second strategy involves reducing the size of the channels between posts by moving from micron-spaced posts to nano-spaced posts, with a somewhat less dramatic decrease in the

post diameter. Increasing the post density requires sophisticated nanopatterning methods,^{6,10–13} which are available for prototyping and somewhat more challenging to implement for mass production. Note that this strategy cannot be extended down to an arbitrarily small gap between the posts; as the post density increases, the transport mechanism will switch eventually from the cyclic rope-over-pulley model^{8,28,29} to biased reptation in a tight (artificial) gel.³⁶ After the cross-over point, the separation will no longer take place as the strong field in a tight gel leads to biased reptation with orientation.

In many cases, such nanopost arrays have been used to separate quite different sizes of DNA at very high rates. For example, the nanopost array of Chan *et al.*⁹ obtained a resolution of 0.8 in 121 s between 21 kbp DNA and 165 kbp DNA, where the latter molecule is much larger than λ -DNA. Similarly, Yasui *et al.*³⁷ reported a remarkable 12 s separation of λ -DNA and 1 kbp DNA in a nanopost array, but in this case the smaller DNA contour length is only a few Kuhn lengths. The separations of binary mixtures of very widely different molecular weights can arise by spanning different migration regimes,¹ which makes comparing these very high quality results to separations of narrower molecular weight regimes more challenging. Indeed, referring back to the pioneering work on nanopost arrays,⁶ it seems from the relative mobility plots (Fig. 5 of Ref. 6) that the molecular weights we studied here would not be effectively separated in a nanopost array.

It is also interesting to compare our results to the so-called “nanofence” array.¹⁹ The latter device uses a lamellar configuration consisting of two offset rows of small posts (500 nm diameter) that are closely spaced (700 nm center-to-center distance) with a large gap (20 μ m) between each of the fences. The operating principle behind the nanofence is that the DNA is forced to collide in each fence, since the spacing between the posts is commensurate with the radius of gyration of the DNA, but the DNA have sufficient time to relax between subsequent collisions. The nanofence thus represents a different way to break up the channels inside an array while preserving the rope-over-pulley separation mechanism. Experiments show that the open region between fences leads to more efficient collisions with the posts than in a hexagonal array.¹⁹ Moreover, Brownian dynamics simulations³⁸ indicate that lamellar configurations are superior to (untilted) hexagonal arrays of similar post density, and the experimental data¹⁹ are in accord with the simulation result. For the XhoI digest, the nanofence array achieved a resolution of 0.91 in 400 s for the 33.5 kbp and 48.5 kbp peaks, which is comparable to the separation in our tilted array of the 20 kbp and 48.5 kbp DNA. The correspondence between the separation resolution in a tilted array and the nanofence array serves to reinforce the general principle that breaking up the channels in the device, while maintaining good collision statistics, is the key to improving separations in post arrays.

Finally, while separation resolution is clearly an important figure of merit, there are some challenges when comparing resolutions reported in different publications for different devices. The separation resolution arises from a number of factors,³¹ in particular the width of the injection band, that are independent of the separation mechanism inside the array. For example, recent experiments³⁹ showed that the separation resolution in a post array is markedly improved using sample-stacking at the inlet to sharpen the initial band. This injection method should, in principle, also improve the separation resolution of our experiments as well. Thus, while separation resolution is clearly the metric of choice for comparing different experiments, making an “apples-to-apples” comparison is not always straightforward. These differences should be kept in mind with respect to our discussion here, especially for the comparison between results from different labs.

B. Comparison with Brownian dynamics simulations

The key simulation data²⁴ suggesting that tilted post arrays would lead to improved separations were obtained using different molecular weights (λ and T4 DNA, 169 kbp) with tilt angles at 10° increments from 0° (Fig. 1(a)) to 30° (Fig. 1(c)). Extensive simulation data were obtained for the low porosity, untilted array (0°) and a tilt angle of 20° for a range of electric fields, using a large number of DNA per ensemble and long travel distances to obtain good statistics. Two key results emerged from this study.²⁴ First, while the separation in the untilted

array degraded as the electric field increases, separations should still be obtained at relatively high electric fields in the tilted array. This prediction is already realized in the experimental data in Fig. 3, where we observe separations in the tilted array up to 50 V/cm while untilted arrays usually lose resolution in the range of 10–20 V/cm.^{4,5,17} Second, the time to achieve a resolution $R = 1$ in a tilted array should decrease almost exponentially as a function of the electric field, albeit with a relatively small prefactor. This particular mode of analysis, namely, fixing the desired separation resolution and then computing the required time/length for the separation, allows us to remove artifacts due to the finite length of the device; the separation is usually faster but lower resolution as the electric field increases for a device of fixed length, which is what we saw in Fig. 3. The idea behind rescaling to a fixed resolution is to evaluate the most important figure of merit (time for the separation) in the context of the constraints imposed by fabrication (the length of the device).

We should also note that the simulation prediction of superior performance in a tilted array²⁴ motivated us to use a smaller molecular weight difference in our experiments than the simulations, which provides a stringent test of the device performance, while at the same time avoiding some of the challenges of loading very large DNA without breakage⁴⁰ (and thus band broadening due to small differences in molecular weight in the sheared DNA). While we have easily separated the 20 kbp and 48.5 kbp molecular weights over a range of electric fields, the particular dependence of the separation performance as a function of field is not readily apparent from the data presented thus far.

In order to test this second prediction from the Brownian dynamics simulations, we need to first recognize that the simulation data correspond to trajectories of individual molecules, and the resolution is thus obtained in the “snapshot” mode at a fixed time using the average velocity and effective diffusion coefficients obtained from these trajectories. In order to compare our finish line resolution data in a 4 mm device to the snapshot data obtained from the simulations as a function of time, we need to make two assumptions. For a snapshot detection,^{2,31} the separation resolution can be expressed in the alternate form

$$R = \frac{\Delta\mu}{\langle\mu\rangle} \sqrt{\frac{N}{16}}, \quad (2)$$

where $\Delta\mu$ is the difference between the mobility of the two species, $\langle\mu\rangle$ is the average of their mobilities, and N is the number of theoretical plates. Since the number of plates is linear in the length L of the device, we have $R \sim \sqrt{L}$. Moreover, we also know that the elution time is proportional to the size of the device, $t \sim L$. As a result, we can rescale our data in Fig. 4 to estimate both the device size and the corresponding time required to reach $R = 1$ if we assume that the snapshot and finish line detection are equivalent. While these scaling relationships are only rigorously true for snapshot detection, they are a reasonable approximation for finish line detection, especially when the residence time in the detector is small.^{2,31}

With the latter assumptions in mind, let us now consider the time and device size estimates for achieving a unit resolution in Fig. 6. The magnitudes of these quantities for our experiments are larger than the typical simulation values²⁴ of $t \approx 60$ s and $L \approx 1$ mm. The quantitative disagreement is entirely expected; the simulations correspond to an easier separation (molecular weight ratio of 4 versus a ratio of 2 in the experiments) and a “perfect” separation apparatus with delta function injection and zero detector width. Nevertheless, it is satisfying to see that simulations predict the correct order of magnitude for t and L in the experiments, even if the systems are somewhat different. This concordance further underscores the quality of the simulation model, as evidenced in previous work.¹⁸

While we expect that the device should continue to reach unity resolution at a reasonable length even up to 50 V/cm, we did not observe the exponential decay in the required time as a function of electric field that was seen in simulations.²⁴ Rather, to within the experimental error, the time required to reach $R = 1$ should be approximately 300 s. This is clearly much improved over the untilted post array, which requires around 10–15 min to reach the same resolution for this range of molecular weights.¹⁷

FIG. 6. Estimates for the time (A) and array length (B) needed to reach a resolution $R = 1$. The projections to $R = 1$ were made for each data point in Fig. 3, and the error bars correspond to the standard deviation of this population of estimates. The light blue sub-bars in (A) are estimates that do not include the contribution to the variance due to the injection, corresponding more closely with the simulation methodology. The error bars are excluded from these second estimates for clarity, but they are similar to the error bars for the dark blue bars.

We might suspect that the deviation between the simulation predictions and our experiments may be attributed, at least in part, to the methodology used in the calculations. Explicitly, the resolution calculations from the simulation only consider the transport inside the array, neglecting contributions due to injection, the finite width of the detector, and other experimentally relevant factors.³¹ In our analysis, we assume that the resolution from our experiment simply scales up like $t^{1/2}$ to the longer array, which includes the initial peak width due to the injection. As a result, we are likely underestimating the performance of a rescaled device when the residence time is small, i.e., for the larger electric fields.

To provide a somewhat more realistic comparison with the simulations, let us denote by σ_e^2 the variance at the end of the finish line experiment for a given electric field, converted from the time domain to the space domain using the mean velocity.² It is common practice to assume that the variances are additive,³¹ so

$$\sigma_e^2 = \sigma_d^2 + \sigma_i^2, \quad (3)$$

where $\sigma_d^2 = 2D^*t$ is the contribution due to the effective diffusivity of strength D^* at the time t of the elution of the peak and σ_i^2 is the band width caused by the finite injection, which we measured in each experiment directly before the DNA entered the array. We can then use Eq. (3) to estimate the effective diffusion coefficient, D^* . We can then estimate the variance of the scaled-up system as

$$\sigma_s^2 = 2D^*t_s + \sigma_i^2, \quad (4)$$

where t_s is the time required to reach a resolution $R = 1$ for the scaled-up channel length. Note that σ_i^2 , the contribution due to injection, and D^* , the rate of band broadening due to transport inside the array, are unchanged by increasing the channel length.

The light-blue bars in Fig. 6(a) show the result of this slightly more realistic analysis. The decrease in separation time as a function of electric field is somewhat more pronounced than our original analysis (dark-blue bars in Fig. 6(a)), but the effect due to injection is not substantial. This is not unexpected. Tight injection bands, along with dissipation of Joule heat, were the major driving force behind microfluidic electrophoresis systems in the first place.⁴¹ As a result, we conclude that the time to reach a resolution of $R = 1$ is indeed independent of electric field, at least to within experimental error.

There are other possible reasons for the disagreement between simulations and experiments. For example, the simulation results correspond to single molecule trajectories, which are used to extract a mean velocity and effective diffusion coefficient for the ensemble, whereas the experiments can involve complicated interactions between the DNA molecules since the suspension is not infinitely dilute. There are also interactions due to roughness of the walls and the posts, as

well as electro-osmotic flow in the channel, that are not included in the simulation model. The impact of these effects on the band broadening would be larger at higher electric fields,⁴² which could be keeping the time to reach $R = 1$ from decreasing as the electric field is increased.

Although the time to reach resolution of unity is about the same for all electric fields, we saw in Fig. 4 that running the device at higher electric fields does seem to increase the reproducibility of the separation. However, larger electric fields require longer separation channels to reach high resolution in the tilted post array. For integrated lab-on-a-chip devices with many operations, space is at a premium. Wafers can be up to 300 mm, but that needs to be for the entire device and machines to process 300 mm wafers are not readily available outside semiconductor manufacture facilities. A more standard wafer size for academic research is 100 mm. Even if the device only performs the separation, as opposed to some integrated lab-on-a-chip functionality, we have found that 25 mm is roughly the upper limit for the separation channel since we need to (i) keep the reservoir spacing far enough for pipetting (and avoiding electrical short circuits) and (ii) have the entire pattern far enough away from the edge of the wafer to get sharp walls during photolithography. One option for increasing the channel length is to use serpentine channels,^{43,44} thereby keeping the pattern far from the walls while increasing the length of the channel. We suspect that the increase in band broadening in serpentine channels is not worth the increased length they provide. Thus, one must consider these opposing effects for increasing the electric field when designing a separation device using the tilted post array.

C. Comparison with other tilted systems

Our tilted array strategy, which relies on periodic collisions with the posts and strong deformation of the DNA, differs qualitatively from related work using driving forces at an angle with respect to the lattice vectors of a periodic array. Two notable examples from previous work from the Austin group at Princeton include pulsed field electrophoresis in post arrays⁴⁵ and the DNA prism,⁴⁶ both of which involved alternating electric fields between 0° and 135° to effect either a separation by the switchback mechanism^{45,47} or a continuous separation.⁴⁶ In both cases, the DNA remain in a highly extended state, stretched between the posts, rather than recoiling and then extending again in a DNA-post collision.

Our tilted array strategy also differs from tilted Brownian ratchets.^{48–51} Although the latter devices apply a continuous field at an angle with respect to the lattice vectors of the array, the obstacles in the device are larger than the radius of gyration of the DNA. As a result, DNA in the tilted Brownian ratchet leads to no deformation of the DNA and a qualitatively different separation mechanism.

The deformation of the DNA also makes DNA electrophoresis in a tilted array qualitatively different than separations of colloidal particles⁵² and cells⁵³ in tilted post arrays. In the latter cases, the analytes tend to move at an angle with respect to the driving force, a method known as deterministic lateral displacement.⁵⁴ Since the DNA loses memory of its previous transport upon collision with a post,¹⁸ it is not deflected by the post and thus does not exhibit deterministic lateral displacement. Rather, the separation proceeds in a manner similar to the separation in a conventional post array, with the DNA all moving in the direction of the applied voltage drop.

V. CONCLUSION

We presented results for the separation of 20 kbp DNA and λ DNA in a 4 mm long separation channel using a 15° tilted post array. We were able to achieve measurable separation resolutions in this system for electric fields up to 50 V/cm. This compares favorably with untilted post arrays, which require separation lengths that are typically 15 mm or larger and lose the separation around 20 V/cm.^{4,5,17} However, we did not see the exponential decrease in the time required to achieve a resolution of unity as predicted by simulations.²⁴ Rather, with some reasonable assumptions, we estimate that the separation time will be independent of the electric field strength. We also observed that the DNA separation is more reproducible at higher fields, which could be useful in designing a device to achieve a desired separation.

The prototype tilted post arrays we studied here are promising, and there are two obvious routes for improving the separation resolution. First, we suspect that the resolution would increase if we maintained the same post density but used smaller posts. Ou *et al.* previously showed that thinner posts work very well in improving separations in an untilted array,¹⁷ and there is no reason to expect that the case will be different for an untilted array. Second, improved injection schemes such as sample stacking³⁹ could reduce the width of the injection band and thus further increase the resolution. By implementing these improvements into the tilted post array, it may be possible to achieve rapid base line separations that could be very useful in an integrated lab-on-a-chip device.

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