Separation of DNA by length in rotational flow: Lattice-Boltzmann-based simulations

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We use a lattice-Boltzmann based Brownian dynamics simulation to investigate the separation of different lengths of DNA through the combination of a trapping force and the microflow created by counter-rotating vortices. We can separate most long DNA molecules from shorter chains that have lengths differing by as little as 30%. The sensitivity of this technique is determined by the flow rate, size of the trapping region, and the trapping strength. We expect that this technique can be used in microfluidic devices to separate long DNA fragments that result from techniques such as restriction enzyme digests of genomic DNA. © 2015 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4926667>]

The development of novel methods for manipulating biopolymers such as DNA is required for the continued advancement of microfluidic devices. Techniques such as restriction enzyme digests for genomic sequencing rely on the detection of DNA that differ in length by sometimes thousands of base pairs.¹ Methods that separate DNA strands with resolutions on the order of kilobase pairs are required to analyze the products of this technique. To gain an insight into possible techniques to separate polymers, it can be helpful to review the methods to separate particles in microfluidic devices. Experimental work has shown how hydrodynamic mechanisms can lead to separation of particles based on size and deformability.² Eddies, microvortices, and hydrodynamic tweezers have been used to trap and sort particles. The mechanism of the trapping and sorting arises from the differences between interactions of the particles with the fluid.^{2–8} In particular, counter-rotating vortices have been used to sort particles and manipulate biopolymers. They have been used to deposit DNA precisely across electrodes⁹ and trap DNA.^{10,11} Vortex flow may therefore be a good basis for a technique for sorting DNA by length.

Streaming flow has been used in experiments to separate colloids of different size.^{3,4} Particles are passed through a channel with a flow field driven by oscillating bubbles and pressure. The flow field becomes a combination of closed and open streamlines. The vortex flow is controlled by the acoustic driving of the bubbles while pressure controls the net flow of the fluid. Larger particles are trapped in the closed vortex flow created by the bubbles, while smaller particles can escape the neighborhood of a bubble in the open streamlines. This leads to efficient separation of particles with size differences as small as 1 μm .

Previous work on DNA has shown that counter-rotating vortices can be used to trap DNA dynamically. Long strands of DNA have been observed to stretch between the centers of two counter-rotating vortices. The polymer stays trapped in this state for significant amounts of time.¹² In a different experiment, the vortices were used to thermally cycle the polymer and allow replication via the polymerase chain reaction (PCR). The DNA is also trapped against one wall by a thermophoretic force in these experiments.¹⁰ The strength of the trap is controlled by the gradient in temperature created by a focused infrared laser beam.

Trapping DNA at one wall by counter-rotating vortices has also been explored in simulation and found to depend on the Peclet number, $Pe = u_{\text{max}}L/D_m$, where u_{max} is the maximum speed of the vortex, L is the box size, and D_m is the diffusion coefficient of one bead in the

polymer chain.¹¹ The trapping rate of the DNA was shown to depend on the competition between the flow compressing the DNA into the trap region and the diffusion of the DNA out of the trap. For the work presented here, $Pe \cong 2000$, similar to the previous work done with the same simulation.

We extend the previous work to investigate if counter-rotating vortices can be used to separate DNA of different lengths. We use the same type of simulation outlined in Refs. 11 and 13–17, based on the lattice-Boltzmann method. The simulation method has successfully modeled systems as diverse as thermophoresis of DNA,¹⁴ migration of DNA in a microchannel,¹⁶ and translocation of DNA through a micropore.^{17,18} Using this method, the fluid is broken into a lattice with size, ΔL , chosen to be $0.5 \mu\text{m}$, and is coupled to a worm-like chain model with Brownian dynamics for the polymer.^{19,20} The fluid velocity distribution function, $n_i(\mathbf{r}, t)$, describes the fraction of fluid particles with a discretized velocity, $\mathbf{c}_i$, at each lattice site.^{21–24} A discrete velocity scheme with nineteen different velocities in three dimensions is used. The velocity distributions will evolve according to

$$n_i(\mathbf{r} + \mathbf{c}_i \Delta \tau, t + \Delta \tau) = n_i(\mathbf{r}, t) + \mathbf{L}_{ij}[n_j(\mathbf{r}, t) - n_j^{eq}(\mathbf{r}, t)], \quad (1)$$

where $\mathbf{L}$ is a collision operator such that the fluid relaxes to the equilibrium distribution, n_i^{eq} given by a second-order expansion of the Maxwell-Boltzmann distribution

$$n_i^{eq} = \rho a^{c_i} [1 + (\mathbf{c}_i \cdot \mathbf{u})/c_s^2 + \mathbf{u} \mathbf{u} : (\mathbf{c}_i \mathbf{c}_i - c_s^2 \mathbf{I})/(2c_s^4)], \quad (2)$$

where $c_s = \sqrt{1/3 \Delta L \Delta \tau}$ is the speed of sound. $\Delta \tau$ is the time step for the fluid in the simulation, $\Delta \tau = 8.8 \times 10^{-5}$. The coefficients a^{c_i} are determined by satisfying a local isotropy condition

$$\sum_i a^{c_i} \mathbf{c}_{i\alpha} \mathbf{c}_{i\beta} \mathbf{c}_{i\gamma} \mathbf{c}_{i\delta} = c_s^4 (\delta_{\alpha\beta} \delta_{\gamma\delta} + \delta_{\alpha\gamma} \delta_{\beta\delta} + \delta_{\alpha\delta} \delta_{\beta\gamma}). \quad (3)$$

To simplify computation, the velocity distributions are transformed into moment space. The density ρ , momentum density $\mathbf{j}$, and momentum flux density $\mathbf{\Pi}$ are some of the hydrodynamic moments of $n_i(\mathbf{r}, t)$. The equilibrium conditions for these three moments are given by

$$\rho = \sum_i n_i^{eq}, \quad (4)$$

$$\mathbf{j} = \sum_i \mathbf{c}_i \cdot n_i^{eq}, \quad (5)$$

$$\mathbf{\Pi} = \sum_i n_i^{eq} \cdot \mathbf{c}_i \mathbf{c}_i. \quad (6)$$

$\mathbf{L}$ has eigenvalues $\tau_0^{-1}, \tau_1^{-1}, \dots, \tau_{18}^{-1}$, which are the characteristic relaxation times of the moments. The Bhatnagar-Gross-Krook model is used to determine $\mathbf{L}$:²⁵ the non-conserved moments have a single relaxation time, $\tau_s = 1.0$. The conserved moments are density and momentum; for these, $\tau^{-1} = 0$. Fluctuations are added to the fluid stress as in the method of Ladd.²⁴ We have also compared simulations with lattice sizes of $1 \mu\text{m}$ and $0.25 \mu\text{m}$ and found no significant differences in the results.

The DNA used in the simulation is represented by a worm-like chain model parameterized to capture the dynamics of YOYO-stained λ DNA in bulk solution at room temperature.^{15,16,26} Long, flexible DNA is modeled since techniques to separate long DNA molecules with kilobase pair resolution are necessary to complete techniques such as genomic level sequencing using restriction enzyme digests.¹ In addition, such DNA is often used in experiment. Its properties are similar to unstained DNA or DNA stained by other methods.²⁷ Each molecule is represented by N_b beads and $N_b - 1$ springs. A chain composed of $N_b - 1$ springs will have a contour length of $(N_b - 1) \times 2.1 \mu\text{m}$. The forces acting on each monomer include: an excluded volume force, a non-linear spring force, the viscous drag force, a random force that produces Brownian motion,

a repulsive force from the container walls, and an attractive trapping force only at one wall as shown in Fig. 1.¹³ The excluded volume interaction between beads i and j located at $\mathbf{r}_i$ and $\mathbf{r}_j$ is modeled using the following potential:

$$U_{ij}^{ev} = \frac{1}{2} k_B T \nu N_{ks}^2 \left(\frac{3}{4\pi S_s^2} \right) \exp \left(\frac{-3|r_i - r_j|^2}{4S_s^2} \right), \quad (7)$$

where $\nu = \sigma_k^3$ is the excluded volume parameter with $\sigma_k = 0.105 \mu\text{m}$, the length of one Kuhn segment, $N_{ks} = 19.8$ is the number of Kuhn segments per spring, and $S_s^2 = N_{ks}/6 \sigma_k^2$ is the characteristic size of the bead. This excluded volume potential reproduces self avoiding walk statistics. The non-linear spring force is based on force-extension curves from experiments and is given by

$$\mathbf{f}_{ij}^s = \frac{k_B T}{2\sigma_k} \left[\left(1 - \frac{|\mathbf{r}_j - \mathbf{r}_i|}{N_{ks}\sigma_k} \right)^{-2} + 4 \frac{|\mathbf{r}_j - \mathbf{r}_i|}{n_K \sigma_k} - 1 \right] \frac{\mathbf{r}_j - \mathbf{r}_i}{|\mathbf{r}_j - \mathbf{r}_i|}, \quad (8)$$

which applies when $N_{ks} \gg 1$.

The beads are modeled as freely draining but subject to a drag force given by

$$\mathbf{F}_f = -6\pi\eta a(\mathbf{u}_p - \mathbf{u}_f). \quad (9)$$

The beads are also subjected to a random forcing term that is drawn from a Gaussian distribution with zero mean and a variance

$$\sigma_v = 2k_B T \zeta \Delta t. \quad (10)$$

The random force reproduces Brownian motion. To conserve total momentum, the momentum change imparted to the beads through their interactions with the fluid is balanced by a momentum change in the fluid. The momentum change is distributed to the three closest fluid lattice sites using a linear interpolation scheme based on the proximity of the lattice site to the polymer beads. Through this momentum transfer, hydrodynamic interactions between the beads occur.

The beads are repelled from the walls with a force of magnitude

$$F_{wall} = \frac{250k_B T}{\sigma_k^3 (x_{bead} - x_{wall})^2}, \quad x_{bead} > (x_{wall} - 1), \quad (11)$$

FIG. 1. Simulation set-up. Arrows indicate direction of fluid flow. The region where the trapping force is active is shaded, and its width (X_{stick}) is shown. The region used to determine the trapping rate is indicated by the area labeled trap region. Figure is not to scale, the trap region and X_{stick} are smaller than shown.

where the repulsion range is $1\Delta L$. Each monomer will also be attracted to the top wall by a force with magnitude

$$F_{stick} = \frac{K_{stick}k_B T}{\sigma_k^3(x_{bead} - x_{wall} + 10)^2}, \quad x_{bead} > (x_{wall} - X_{stick}) \quad (12)$$

and range X_{stick} (see Fig. 1). The sticking force is turned off every one out of one hundred time steps of the polymer (1% of the simulation time steps). We vary both X_{stick} and K_{stick} to achieve separation of the polymers.

In previous experiments, DNA has been trapped against one wall by using thermophoresis,¹⁰ dielectrophoresis,²⁸ and nanoplasmonic tweezers.²⁹ In the case of thermophoresis, the trap strength (K_{stick}) can be controlled by tuning the intensity of the temperature gradient and the trap extension (X_{stick}) can be controlled through the area over which the gradient extends. Both of these are set through focusing of the laser used to produce local heating. Similarly, the trap parameters can be controlled when using plasmonic tweezers by controlling the laser beam exciting the nanoplasmonic structures. In dielectrophoresis, the DNA is trapped by an AC electric field and can be controlled by tuning the frequency and amplitude of the field.

In this work, the number of polymers, N_p , is 10 unless otherwise noted, and the container size is $25\Delta L \times 50\Delta L \times 2\Delta L$. The time step for the fluid is $\Delta\tau = 8.8 \times 10^{-5}$ s, and for the polymer is $\Delta t = 3.7 \times 10^{-6}$ s. The total simulation time is over 100 chain relaxation times, allowing sufficient independent samples to perform statistical analysis.

Two counter-rotating vortices, shown in 1, are produced by introducing external forces to the fluid bound by walls in the x -direction and periodic in the y and z . Two forces of equal magnitude push on the fluid in the upper x region ($12\Delta L < x < 25\Delta L$): one in the $+y$ -direction along $y = 10\Delta L$, and one in the $-y$ -direction along $y = 40\Delta L$. Such counter-rotating vortices can be produced in microfluidic channels using acoustically driven bubbles,^{3,4,30} local heating,¹⁰ or plasmonic nanostructures.⁵ The flow speed is controlled by very different external mechanisms in each case. We therefore choose a simple model to produce fluid flow that is not specific to one mechanism.

The simulations are started using random initial conditions, and therefore, both lengths of polymer are dispersed throughout the channel. Within a few minutes, the steady state configurations pictured in Figs. 2 and 3 are reached. We define the steady state as when the number of polymer chains in the trap changes by less than one chain (10 beads) per 1000 polymer time steps. Intermittently, some polymers may still escape and re-enter the trap even in the steady state. Three final configurations are possible: Both the lengths of DNA have become trapped, both lengths continue to rotate freely, or the shorter strand has become trapped while the longer rotates freely. Two of these states leave the polymers mixed; in the third, the strands have separated by size.

By tuning the attractive wall force parameters and fluid flow, the separated steady state can be realized. We first set the flow parameters that allow the larger chains to rotate freely at the center of the vortices while the shorter chains rotate closer to the wall. The trap strength, K_{stick} , and extension, X_{stick} , are changed until the shorter polymers do not leave the trap. The same parameters were used to separate 10-bead chains from 15-bead and 13-bead chains.

As shown in Fig. 2, we have been able to separate shorter 10-bead chains from longer 15-bead chains. In the steady state, 97% of the rotating polymers were long polymers averaged over twenty simulations initialized with different random starting conditions. For three simulations, one small polymer would intermittently leave the trap region. In two of these simulations, one long polymer became stably trapped in the steady state. In another simulation, one 15-bead chain was intermittently trapped. On average, the trapped polymers were 5% 15-bead chains and 95% 10-bead chains. Again, 97% of the rotating polymers were 15-bead chains.

Simulations conducted with 10-bead and 13-bead chains also showed significant separation of the two sizes as can be seen in Fig. 3. In the steady state, 30% of the trapped polymers are 13-bead chains and 70% are 10-bead chains, averaged over twenty different random initial

FIG. 2. Snapshots at $t = 0\Delta t$ (left) and $t = 2500\Delta t$ (right) showing the separation of 15-bead strands (grey) from 10-bead strands (black) of DNA. For these simulations, $K_{stick} = 55$ and $Y_{stick} = 0.7\Delta L$.

FIG. 3. Snapshots at $t = 0\Delta t$ and $t = 2500\Delta t$ showing the separation of 13-bead strands (grey) from 10-bead strands (black) of DNA. For these simulations, $K_{stick} = 55$ and $Y_{stick} = 0.7\Delta L$ as in Fig. 2. Note that one long polymer is trapped, as well as all of the shorter polymers.

starting conditions and 1000 polymer time steps. Only 14.8% of the shorter polymers were not trapped, leading to 85.2% of the freely rotating chains being 13-bead chains. This is therefore a viable test to detect the presence of these longer chains.

We have also separated 20-bead chains from 10-bead chains with all of the shorter chains trapped and all of the longer chains freely rotating in the steady-state. These results do not change for twenty different random initial starting conditions and 1000 polymer time steps. None of the longer polymers intermittently enter the trap region nor do any of the shorter polymers intermittently escape.

The separation is achieved by tuning the trapping force and flow rate. Strong flows will push all the DNA molecules into the trap. The final state is mixed, with both short and long strands trapped. For flows that are too weak, the short molecules are not sufficiently compressed by the flow and therefore do not enter the trap region. The end state is mixed, with all polymers freely rotating. Separation is achieved when the flow rate is tuned so that the short strands are compressed against the channel wall while the long polymers rotate near the center of the vortices. The trap strength must then be set sufficiently high enough to prevent the short strands from being pulled by the hydrodynamic drag force out of the trap.

The mechanism of the separation depends on the differences in the steady state configurations of the polymers and chances of a polymer escaping the trap. As shown in Fig. 4, both longer and shorter chains are pulled into the trap region by the flow. However, the longer chains have a larger chance of a bead escaping into a region of the flow where the fluid velocity is sufficient to pull the entire strand out of the trap. As shown in Ref. 11, the trapping rate depends on diffusion in a polymer depleted region near the trap, in agreement with classical theory which neglects bead-wall interactions. In addition, the theory depends on the single bead diffusion rate and does not take into account the elastic force holding the beads together. Diffusion becomes as significant as convection in the polymer depleted region leading to dependence on the Peclet number. Since longer polymers have more beads; they have more chances of a single bead diffusing through this layer into the region where convection is again more important. Thus, they are pulled out of the trap at a faster rate than the shorter chains.

In addition, longer chains have a second trap resulting from the microflow. As shown in Ref. 12, DNA in counter-rotating vortices can tumble at the center of one vortex or be stretched between the centers of the two vortices. We have observed both these conformations for the longer polymer strand. They are a stable trajectory for the longer polymer that remains outside of the trapping region. As seen in Fig. 4, few monomers of the longer chains enter the trap region once the steady state has been reached. However, the shorter polymer rotates at a larger radius than the longer polymer as seen in Fig. 5. The shorter polymers therefore are pushed back into the trap while the longer strands rotate stably outside the trapping region.

This mechanism is similar to the one proposed for the separation of colloids by size in Refs. 3 and 4. In that experimental work, the smaller colloidal particles rotated at larger radii. This allowed the smaller beads to be pushed out of the vicinity of the vortices by the streaming flow, while the larger beads continued to circle. However, in our simulations, we have the additional mechanism of separation based on the increased chance of a longer polymer escaping the trap region. This mechanism is important for maintaining the separation. Long polymers initially in the trap region or which diffuse into the trap would not be able to escape without it.

We expect that this technique could be used to detect the sizes of DNA fragments on the order of thousands of base pairs. It relies on the flexibility of the molecule and its interaction with the flow. Common lab procedures such as restriction enzyme digests for DNA fingerprinting can produce these long fragments. Current techniques such as gel electrophoresis require significant time to separate the long strands that move more slowly through the matrix. This effect could therefore be a good candidate for developing a microfluidic analysis that is significantly faster than traditional procedures. Our separation occurs in minutes rather than in hours as for gel electrophoresis.

As pointed out in Ref. 2, hydrodynamic effects have been shown to be important for microfluidic devices for separation. We have demonstrated, in simulation, a novel hydrodynamic

FIG. 4. N , number of beads in the trap region, versus time for 15-bead DNA strands (solid line) and 10-bead DNA strands (dashed line). Here, $\Delta T = 10000\Delta t$. The simulation parameters are the same as in Fig. 2.

FIG. 5. Trajectories of 15-bead DNA (grey) and 10-bead DNA (black). The position of each monomer is plotted for 100 consecutive time steps. Note that the longer polymers rotate in the center of the channel while the shorter polymers rotate at the edges. Simulation parameters are the same as in Fig. 2.

mechanism for separating polymers by length. We hope that these promising calculations will inspire experiments to verify these results.

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