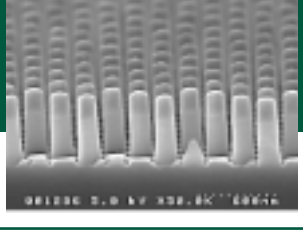
MESA MONOGRAPHS

Micro Total Analysis Systems 2003



Proceedings of μTAS 2003
Seventh International Conference
on Micro Total Analysis Systems

October 5 – 9, 2003
Squaw Valley, California, USA

VOLUME 1 & 2

ISBN: 0-9743611-0-0
TRF CATALOG NUMBER: 03TRF-0001

EDITORS:
M. Allen Northrup
Klavs F. Jensen
and D. Jed Harrison

ENHANCED BROWNIAN RATCHET ARRAY FOR DNA SEPARATION USING FLOW ANGLE EFFECT

Lotien Richard Huang[1], James C. Sturm[1], Robert H. Austin[2] and Edward C. Cox[3]

[1]Department of Electrical Engineering, [2]Department of Physics, [3]Department of Molecular Biology, Princeton University, Princeton, NJ 08544, USA.

Abstract

Under strict control of ion flows, we examine the effect of the flow direction with respect to the array on the separation efficiency. Devices for flow angles of 0°, 4°, 8° and 12° are made. The separation angle between bands of 48.5 kb and 164 kb DNA molecules is maximized around 8°, for the specific array design. The enhanced performance of the Brownian ratchet array implies that the array could potentially be a useful device for DNA separation in the ~100 kb range.

Keywords: DNA separation, Brownian ratchet, microfluidic, fractionation

1. Introduction

Separation of analytes is a fundamental technique in many fields, including chemistry, biology, biomedicine, industry and environment control. Separation of DNA molecules larger than 40,000 base pairs according to size plays a key role in many genome projects. The standard gel electrophoresis, using acrylamide or agarose gel, is incapable of separating molecules larger than 40 kb. Large DNA fragments are conventionally separated by pulsed-field gel electrophoresis (PFGE), which however, is extremely time-consuming, with running times of typically more than 10 hr. The Brownian ratchet array reported here, enhanced by a small flow angle, separates DNA molecules in the ~100 kb range faster than PFGE.

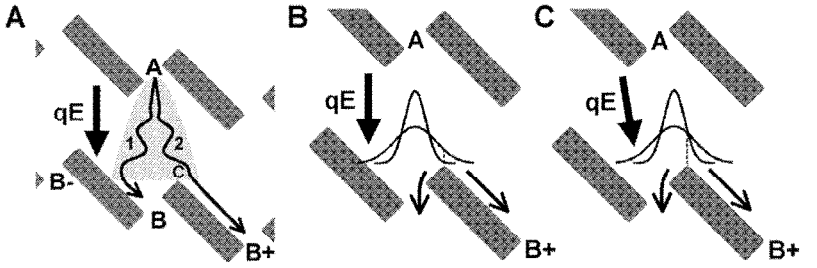


Fig. 1. Basic principle of Brownian ratchet array.

2. Theory

Brownian ratchets, structures that permit Brownian motion in one direction only, have been implemented using micromachining techniques [1, 2]. When particles flow through an asymmetric array of obstacles (Fig. 1A), they are preferentially deflected to the right, because particles moving to the left (path 1) are blocked and deflected back to gap B, whereas those diffusing to the right (path 2) are swept to gap B+. The probability of deflection depends on the amount of diffusion (Fig. 1B). Small molecules spread out more than large molecules and thus have a higher probability of being deflected to gap B+. Different molecules in average migrate along different directions as they flow down the array, according to their diffusion coefficients. The major advantage of the Brownian ratchet array is that it does not require stretching of the molecule—— molecules of any shape can be sorted according to their diffusion coefficients. The Brownian ratchet array is not limited to separating DNA molecules only, but in principle can be applied to sort proteins, organelles, colloids and other molecules of interest.

Previous experiments show that the separation efficiency of the Brownian ratchet array is low [2], and the array is not useful for fractionating DNA molecules of ~100 kb. With a running time of ~2 hr, the resolution between the peaks of 48.5 kb and 164 kb molecules are only 1.4. In this paper, we report an order-of-magnitude improvement in separation speed, by tilting the flow at a small angle with respect to the array.

The basic mechanism for enhancing the separation using the Brownian ratchet array is depicted in Fig. 1B and 1C. When the flow is aligned to the array, the probability of a molecule being deflected to gap B+ is small (Fig. 1B). This results in the low separation efficiency. Now, with the flow tilted at a small angle with respect to the array, the probability of a molecule being deflected to gap B+ is increased, even though the amount of diffusion is the same (Fig. 1C). Without tilting, the separation angle between 48.5 kb and 164 kb DNA molecules at a flow speed of ~1.5 μm/s is ~1.3° [2]. In comparison, with a 8° tilting, the separation angle at the same flow speed reaches ~8°, a factor of 6 larger.

3. Experimental

To optimize the device performance, the direction of the ion flow are carefully controlled. Individual devices are made for different flow angles of 0°, 4 °, 8° and 12°, respectively. The ion flow direction in the array is in general different from the electric field direction, because currents are impeded to different extent by the insulating obstacles at different directions. Therefore, to create current in the desired direction, we have to apply the equi-potential contours at the correct orientation, which has to be calculated numerically by solving Laplace equation using proper boundary conditions. Three features are incorporated in the device design to control the current direction (Fig. 2):

(i) The array is much longer than it is wide.

(ii) The top and bottom edges of the array are slanted to fit the pre-determined equi-potential contours, in order to apply the correct field orientation at the array edges.

(iii) Microfluidic channels sandwiching the array act as electric resistors. This reduces the residual distortion of the current distribution near the top and bottom edges.

4. Results and discussion

A mixture of coliphage λ DNA (48.5 kbp, ~2 μg/ml) and coliphage T2 DNA (164 kb, ~2 μg/ml) in Tris-Borate-EDTA buffer (0.5x) was injected into the array at various speeds using electric fields. The buffer contained 1% POP-6, a performance optimized linear polyacrylamide (Perkin-Elmer Biosystems) to reduce electro-osmotic flow. At high migration speeds (> 4 μm/s) and zero flow angle, the two species of DNA molecules migrate at the same direction along the array [2]. As the flow speed is reduced to ~3 μm/s, the small molecules (48.5 kb) start to separate from the larger ones (164 kb) (Fig. 3). At ~1.5 μm/s, a flow speed that requires ~2 hr of running time through a 12 mm-long array, the separation angle between the two species reaches 1.3°, with a resolution of ~1.4 between the two peaks at the end of the array [2].

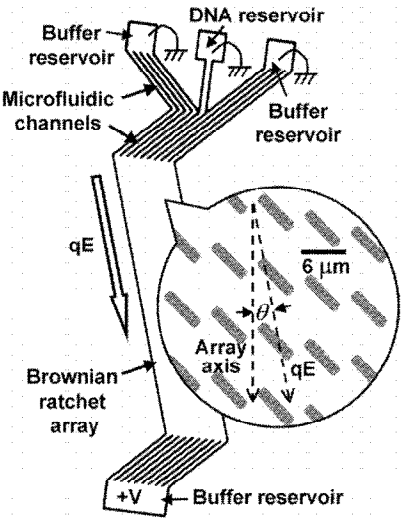


Fig. 2. Schematic diagram of the device at the flow angle of $\theta = 12^\circ$ with respect to the array axis. Devices of $\theta = 0^\circ$, 4° and 8° are also made with similar designs.

Similar samples are injected into devices that are designed at flow angles of 4°, 8° and 12°. The separation between the two species of DNA molecules is increased as we increased the flow angle to 4° and to 8°. The separation angle is shown in Fig. 3, along with the theoretical prediction proposed by our previous work [1]. However, as the flow angle is increased to 12°, the separation becomes small. Although the underlying mechanism is not understood, we speculate that it is due to the insufficient averaging of field lines of DNA molecules at gaps between the obstacles, and all molecules tend to follow the fields regardless of their sizes.

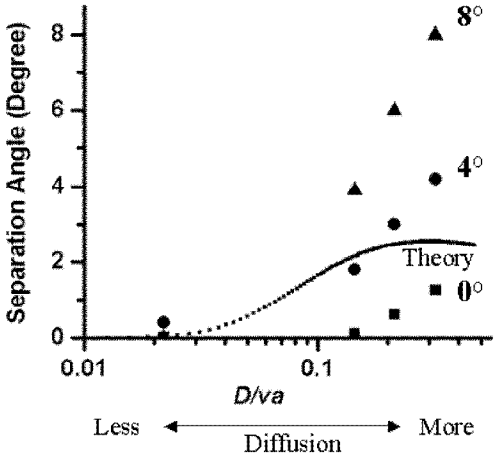


Fig. 3. Measured separation angle as a function of flow velocity and flow angle. The curve is the theory prediction assuming uniform flow. D is the diffusion coefficient, v is the flow velocity, and a is the gap size between the obstacles (1.4 μm).

5. Conclusions

Adjusting the flow direction with respect to the array is an effective way to enhance the performance of Brownian ratchet arrays. Because the probability of a molecule being deflected increases as we tilt flow with respect to the array, the separation between molecules of different sizes is greatly improved. This allows for faster separation and higher resolution. Compared to the previous experiments, which requires ~2 hr of running time to baseline resolve 48.5 kb from 164 kb DNA molecules (resolution = 1.4), the new results at a flow angle of 8° with respect to the array shows separation of the two species in ~1 hr with resolutions as high as ~4. The Brownian ratchet array enhanced by a small flow angle is now useful for DNA separation in the ~100 kb range. Further, because the Brownian ratchet array does not require stretching of molecules, it can in principle separate other biologically important molecules according to their diffusion coefficients.

Acknowledgements

This work was supported by grants from DARPA (MDA972-00-1-0031), NIH (HG01506), and the State of New Jersey (NJCST 99-100-082-2042-007).

References

1. T. A. Duke, R. H. Austin, Phys. Rev. Lett. 80, 1552, (1998)
2. L. R. Huang et. al., Phys Rev Lett 89, 178301 (2002)