DNA Separation Using Photoelectrophoretic Traps

Avital Braiman
Division of Engineering, Brown University,
Providence, RI 02912, USA

Thomas Thundat
Department of Chemical and Materials Engineering,
University of Alberta, Edmonton, AB T6G 2V4, Canada

Fedor Rudakov
Computer Science and Mathematics Division
Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA

ABSTRACT

In our recent publications we presented a design that allows formation of highly localized and optically controlled electrophoretic traps.[1,2] We demonstrated that electrophoretic traps can be utilized for biomolecule photoconcentration, optically directed transport, and separation by size.[1,2] In the current publication we suggest a hybrid design for biomolecule separation which implements electrophoretic traps in tandem with well-established electrophoretic techniques. We perform Monte Carlo simulations that demonstrate that the resolution of well-established electrophoretic techniques can be greatly enhanced by introducing photoelectrophoretic traps.

Keywords: DNA separation, photoelectrophoresis, optical trapping, biomolecules

Separation of biomolecules according to their sizes is required for many biomedical applications. Typically, separation is achieved by electrophoretic transport of charged biomolecules through a sieving media such as gel[3], polymer solution[3,4,5] or microfabricated structures[6,7,8,9] using a uniform electric field. This approach, however, provides limited experimental flexibility: In order to separate biomolecules of a particular size complete separation of the entire sample by size is required. Furthermore, biomolecules migrating through a sieving media are subjected to diffusion resulting in band broadening. In our recent publication we suggested a design for biomolecule separation that permits selective separation of biomolecules from a mixture (complete separation of the sample by size is not required).[1] Selective separation of biomolecules is performed by employing highly localized and optically controlled electric field to transport the biomolecules through a sieving media.

Optically controlled forces are usually created using techniques such as optical and optoelectronic tweezers. Those techniques are well suited for trapping and manipulation of micron sized particles such as cells.[10,11,12,13,14] For smaller particles such as DNA and protein molecules, forces induced by optical and optoelectronic tweezers are small and DNA motion is mostly governed by Brownian fluctuations.[12,15] Manipulation of small particles is achieved by their prior attachment to larger particles.[11,13,14]

Recently we presented a design that allows trapping and manipulation of small changed particles such as protein and DNA molecules without their prior attachment to larger particles.[1,2] Our experimental setup (figure 1) is similar to that of optoelectronic tweezers. Yet, in our design electrophoretic force rather than dielectrophoretic is employed to trap and manipulate the particles. Unlike dielectrophoretic forces, electrophoretic forces can be quite significant for small charged particles and can readily overcome Brownian fluctuations. We call this technique "Photoelectrophoretic Localization and Transport (PELT)".

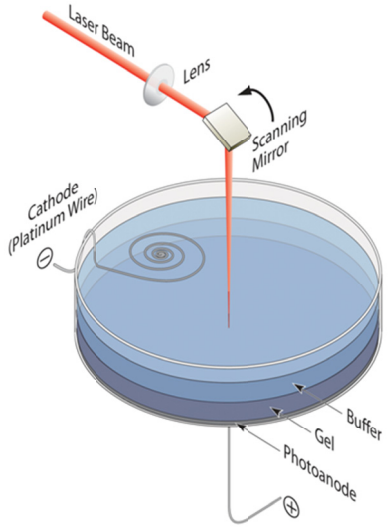

Figure 1 Schematic of the experimental setup. A laser beam focused onto a photoanode topped with gel and buffer generates photocurrent at the illumination spot. Photocurrent results in a highly localized electric field within the electrolyte medium causing negatively charged biomolecules to migrate towards the center of the illumination spot. Moving the focus of the beam along the photoelectrode consequently moves the trap and causes translocation of the trapped biomolecules within the medium.

Our setup is described in details elsewhere.[1] Briefly, a sample is placed in a well in agarose gel that is in contact with a photoanode and topped off with buffer. The photoanode is connected to the counterelectrode through an external circuit. We employ an n-type semiconductor as a photoanode. A potential applied between the photoanode and the counterelectrode placed in the buffer depletes electrons from the surface layer of the semiconductor. In the dark the depletion layer prevents the current from flowing across the semiconductor/electrolyte interface. At the illumination spot the electrons get promoted from the valence band to the conduction band. The charge carriers get separated in the semiconductor's space charge region: the electrons move into the bulk of the

photoelectrode and the holes migrate to the semoconductor's surface where they accept electrons from the molecules of the electrolyte. Thus, a localized photocurrent is produced at the photoillumination spot.

Generated photocurrent within an electrolyte medium results in an electrical field trap according to Ohm's law:

$$\vec{E} = \rho \cdot \vec{j} \quad (1)$$

where ρ is the resistivity and $\vec{j}$ is the vectorial current density in the electrolyte medium. Charged biomolecules within the medium electrophoretically migrate toward the center of the photoelectrophoretic trap. Moving the illumination spot along the photoelectrode translocates the trap and consequently results in migration of the trapped biomolecules within the medium.

In our recent publications we demonstrated that PELT can be used for concentration, manipulation, isoelectric positioning of amphoteric biomolecules, and selective separation of biomolecules of a particular size from a mixture.[1,2] Selective separation of biomolecules is accomplished by manipulation of the speed of the trap. The separating principle is based on the capability of the biomolecules to keep up with the trap. Initially the speed of the trap is set such that the biomolecules of interest could follow the trap, while the next largest in size biomolecules would be incapable of keeping up with the trap. The speed of the photoelectrophoretic trap is then increased, allowing biomolecules of interest to drop out of the trap while the other biomolecules would continue to translocate with the trap. Thus, the biomolecules of interest can be selectively separated from a mixture without performing complete separation of the sample by size.

A major limitation of DNA separation using PELT technique is the long separation time. This limitation arises from two major issues: First oxidation of water at the photoanode results in the generation of H^+ according to:

$$H_2O - 2e^- \rightarrow 2H^+ + \frac{1}{2}O_2 \quad (2)$$

The pH in close proximity to the photoillumination spot is greatly reduced compared to the pH in the bulk solution; thus the charges (and as a consequence the mobilities) of the biomolecules are reduced resulting in increased photoconcentration and separation times. Secondly, the potential between the photoanode and the counterelectrode is limited by the fact that at high potentials biomolecules can get oxidized at the contact with the photoanode.

In the current publication we suggest a hybrid design for biomolecule separation which utilizes PELT in combination with well-established electrophoretic techniques. PELT is utilized for biomolecule concentration, while separation is achieved by electrophoretic transport of the biomolecules using a uniform electric field. The proposed design shown in figure 2 consists of two electrodes ***A*** and ***B***, a non-conductive photoelectrode ***C*** placed in the electrolyte, and an array of lasers. Photoelectrode ***C*** is in contact with a sieving media such as a gel or an array of nanofabricated structures. The laser is focused on the photoelectrode near cathode ***B*** allowing the biomolecules to concentrate at the illumination spot. Then the laser is turned off and a voltage is applied between electrodes ***A*** and ***B***, causing biomolecule translocation towards the anode along photoelectrode ***C***. Upon separation of the biomolecules the potential between electrodes ***A*** and ***B*** is turned off and the laser beams are focused on each individual band, thus causing photoconcentration of biomolecules. Separation thus includes two photoconcentration steps and results in an array of highly concentrated molecular packets, each containing biomolecules of one particular size. As we previously demonstrated in our research, PELT can be employed to independently manipulate the molecular packets in two dimensions.[2]

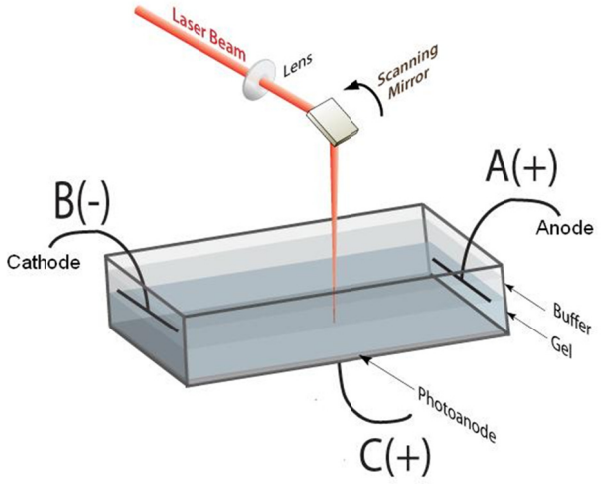

Figure 2 Hybrid design for DNA separation utilizing a uniform electric field and photoelectrophoretic traps to transport the biomolecules. Photoelectrode ***C*** is topped with gel. Prior to performing separation the laser beam is focused on the photoelectrode ***C*** near cathode ***B*** (no potential is applied between electrodes ***A*** and ***B***). The DNA fragments become photoconcentrated at the illumination spot. The laser is then turned off and a potential is applied between electrodes ***A*** and ***B***, inducing DNA translocation towards anode ***A***. Upon translocation through the gel, DNA fragments become separated by size. After separation, the DNA fragments can be photoconcentrated again. To photoconcentrate the DNA fragments the potential between electrodes ***A*** and ***B*** is turned off and the laser is focused at the position of the band that needs to be concentrated.

The paper is constructed in the following way: First, we provide a description of our model. We then perform a rough estimation of the parameters of the photoelectrophoretic trap based on experimental data. The parameters of the photoelectrophoretic trap are then utilized in numerical simulations that demonstrate improvement of resolution of well-established DNA separation techniques when PELT is implemented in the setup.

Model Description

We performed numerical simulations of the translational motion of DNA fragments subject to electrophoretic force and diffusion. The Langevin equation can be employed to describe DNA dynamics.[16,17] We carried out Monte Carlo simulation solving the Langevin equation for DNA fragments translocating in 1.5% agarose gel.

For our simulations we assume that DNA fragments do not interact with each other and are translocating only in horizontal plane. The positions of the DNA fragments are defined by X and Y. During the time interval Δt the DNA fragments consisting of N base-pairs translocate by random distances Δr_x and Δr_y due to diffusion and electrophoretically migrate by a distance $\Delta\overrightarrow{l_N}$. $\Delta r_{x,y}$ satisfy the following conditions:

$$\langle \Delta r_{x,y} \rangle = 0 \quad (3a)$$

$$\langle \Delta r_{x,y}^2 \rangle = 2D_N \Delta t \quad (3b)$$

where D_N is the diffusion coefficient of the DNA fragments consisting of N base-pairs.

$\Delta\overrightarrow{l_N}$ is given by:

$$\Delta\overrightarrow{l_N} = -\mu_N \cdot \vec{E} \cdot \Delta t \quad (4)$$

where μ_N is the electrophoretic mobility of the DNA fragments consisting of N base-pairs and $\vec{E}$ is the electric field created by the potential applied between electrodes $\boldsymbol{A}$ and $\boldsymbol{B}$, or by the photoelectrophoretic trap. We assume that DNA mobilities μ_N and diffusion coefficients D_N are independent of the applied electric field.

The discrete form of the Langevin equation can be written as:

$$X(t+\Delta t) = X(t) - \mu \cdot E_x \cdot \Delta t + \sqrt{2 \cdot D_N \Delta t} \cdot \xi \qquad (5a)$$

$$Y(t+\Delta t) = Y(t) - \mu \cdot E_y \cdot \Delta t + \sqrt{2 \cdot D_N \Delta t} \cdot \xi \qquad (5b)$$

where ξ is sampled from a Gaussian random number distribution with a zero mean ($\langle \xi \rangle = 0$) and a unitary variance ($\langle \xi^2 \rangle = 1$). E_x and E_y are the projections of the electric field on the X and Y coordinates.

Monte-Carlo simulations were performed for 100 bp, 300 bp, 1000 bp, and 3000 bp DNA fragments. The time step Δt was chosen to be 1 s which allows considering electrophoretic forces acting on the DNA fragments to be constant during the integration step. During the time interval of 1s all the internal motion of the DNA molecules is averaged out and is not taken into account in our model. For the smallest DNA fragment (100 bp) the diffusion coefficient was estimated as $D_{100} = 1 \cdot 10^{-7} \frac{cm^2}{s}$ [18][a]. The dependence of the diffusion coefficients on the DNA size was assumed as $D \sim N^{-0.5}$ according to the Zimm model[18]. The mobilities of the DNA fragments at pH=8.3 were estimated as $\mu_{100} = 2.41 \cdot 10^{-4} \frac{cm^2}{V \cdot s}$, $\mu_{300} = 2.04 \cdot 10^{-4} \frac{cm^2}{V \cdot s}$, $\mu_{1000} = 1.31 \cdot 10^{-4} \frac{cm^2}{V \cdot s}$, and $\mu_{3000} = 6.6 \cdot 10^{-5} \frac{cm^2}{V \cdot s}$ according to the experimental data provided in reference [19] for 1.5% agarose gel.

Estimation of the Strength of the Photoelectrophortic Trap

The force exerted by the photoelectrophoretic trap on a DNA fragment depends on multiple parameters. For example, as discussed above, a photocurrent results in accumulation of H^+ ions in close proximity to the illumination spot (equation 2). Thus, as the DNA molecule approaches the center of the photoelectrophoretic trap its charge (and as a consequence mobility) is reduced. Since accumulation of H^+ happens over time, the effective strength of the photoelectrophoretic trap might be time dependent. A detailed analysis should take into account generation of H^+ ions at the illumination spot and their diffusion away from the photoanode. For the purpose of current simulations we perform a rough estimation of the effective potential created by photoelectrophoretic trap from the experimental data. We assume that DNA mobility is independent on its position within the trap and try to find an effective potential that would deliver roughly the same rate and degree of photoconcentration as was experimentally observed.

Photoconcentration of a DNA sample consisting of equal amounts of 3000 bp DNA fragments and 10 bp DNA ladder was performed in 1.5% agarose gel by focusing the laser beam at the right edge of the well containing the DNA sample as described in details in reference [1]. The initial distribution of the DNA fragments is shown in figure 3a. The distribution of the DNA fragments upon photoconcentration for 70 minutes is shown in figure 3b.

In order to determine the parameters of the photoelectrophoretic trap we ran Monte-Carlo simulations solving Langevin equations (5) for a time interval of 70 minutes. The initial distribution of positions of the DNA fragments was chosen according to the fluorescence intensity image shown in figure 3a. Simulations were performed for 1,200,000 100 bp DNA fragments and 1,200,000 3000 bp DNA fragments. The electric field $\vec{E}$ in equations (5) was calculated as $\vec{E} = -\nabla\varphi$, where φ is the electrostatic potential created by photoelectrophoretic trap. The potential φ in the horizontal plane was assumed to be Gaussian:

$$\varphi(r) = A \cdot \exp\left(\frac{-r^2}{2\sigma^2}\right) \qquad (6)$$

where A is the maximum of the potential, $r = \sqrt{X^2 + Y^2}$ is the distance from the center of the trap, and σ is the standard deviation.

Using trial and error method we found that parameters A=0.3 and σ=1 mm provide roughly the same degree and rate of photoconcentration as was experimentally observed. Figure 3c shows computational results for DNA fragment distribution upon photoconcentration for 70 minutes which agree well with the experimental results shown in figure 3b[b]. Since small DNA fragments (<100 bp) have nearly the same dynamics in 1.5% agarose gel, the experimental data (where the 3000 bp DNA fragments and the 10 bp DNA ladder were used) can be directly compared to the simulation results which were performed for the 3000 bp and the 100 bp DNA fragments (the largest DNA fragments in the 10 bp ladder).

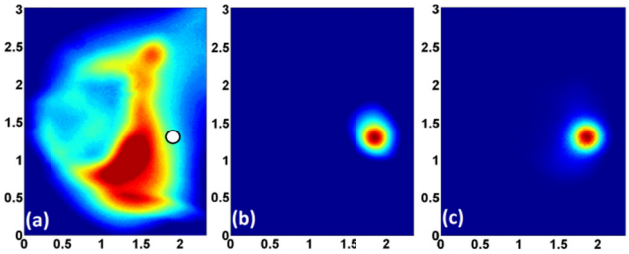

Figure 3 Photoconcentration of a DNA sample consisting of equal amounts of 3000 bp DNA fragments and 10 bp DNA ladder (up to 100 bp). **a**) Initial fluorescence intensity image (false color map). The white spot shows the position of the photoelectrophoretic trap **b**) Fluorescence intensity image of the DNA sample upon photoconcentration for 70 minutes (experimental results). **c**) DNA distribution upon photoconcentration for 70 minutes (computational results). For figure **c** the initial distribution of DNA fragments and the position of the photoelectrophoretic trap was chosen according to figure **a**. The exposure time for figure **a** is 41 sec, for figure **b** is 3 sec.

DNA Separation Using Photoelectrophortic Traps

Having obtained a rough estimation of the parameters of the photoelectrophoretic trap, we perform simulations demonstrating enhancement in resolution of DNA separation by implementing a hybrid design which utilizes electrophoretic traps in tandem with well-established electrophoretic techniques. Simulations were carried out by solving equations (5) for 1,000,000 DNA fragments of each size. The initial positions of the DNA fragments were normally distributed around a point with coordinates [1.5 mm; 1.5 mm] with FWHM =2.35 mm (sigma=1 mm) (figure 4a).

We ran simulations for 3 different cases. The first case corresponds to DNA separation using a uniform electric field to

[a] The diffusion coefficient for 100 bp DNA fragments in 2% agarose gel is 7×10^{-8} cm^2/s according to Figure 3 in reference 18. Our simulations were performed for 1.5% agarose gel. We estimated the diffusion coefficient for the 100 bp fragments in 1.5% gel as 1×10^{-7} cm^2/s.

[b] More accurate estimations of the parameters can be obtained by recording the fluorescence intensity images at multiple times and by using optimization algorithms.

transport the DNA fragments through a sieving media without performing prior photoconcentration. Electrophoresis was performed for 15 minutes with electric field strength E_{unif}=5 V/cm (figure 4b). This case corresponds to DNA separation by well-established electrophoretic techniques and serves for comparison purpose. In the second case, prior to separation, the sample was photoconcentrated for 30 minutes by positioning the trap at [1.5 mm; 1.5 mm] (figure 4c). The initial photoconcentration step allowed reducing the area of the DNA sample to ~1/17 of its original area (FWHM=0.56 mm). The separation thus started from a much smaller sample size and therefore resulted in an increase in resolution. In the third case (figure 4d), the DNA sample was initially photoconcentrated and separated just as in figure 4c. In addition, after separation the DNA fragments in each band were photoconcentrated again resulting in formation of highly concentrated packets of DNA fragments (figure 4d). We performed calculations for a photoconcentration interval of 30 minutes with the positions of the traps set at [12.33 mm; 1.5 mm], [10.69 mm; 1.5 mm], [7.41 mm; 1.5 mm] and [4.47 mm; 1.5 mm]. Photoconcentration resulted in reduction of the width of the DNA bands by approximately a factor of 2.

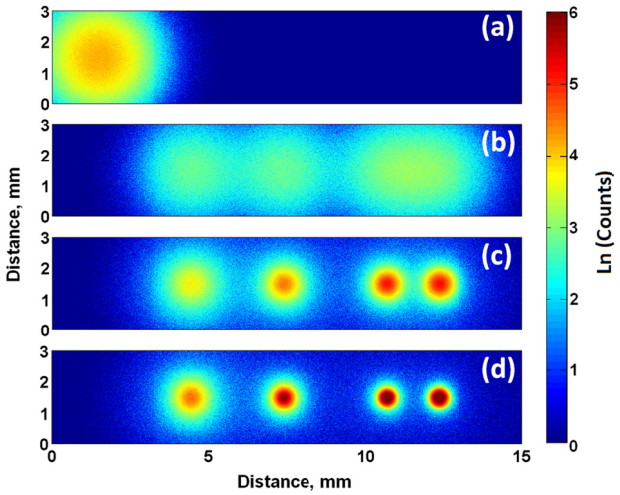

Figure 4 False color map of DNA fragment distribution (logarithmic scale). A DNA sample consists of equal amounts of 100 bp, 300 bp, 1000 bp, and 3000 bp DNA fragments. a) Initial DNA fragment distribution b) DNA fragment distribution upon electrophoretic separation for 15 minutes. c) DNA fragment distribution upon photoconcentration for 30 minutes and electrophoretic separation for 15 minutes. d) DNA fragment distribution upon photoconcentration for 30 minutes, electrophoretic separation for 15 minutes, and photoconcentration of the individual bands for 30 minutes.

In summary, we suggested a design for biomolecules separation which utilizes photoelectrophoretic traps in combination with well-established electrophoretic techniques. The biomolecules are photoconcentrated prior and after performing the separation which allows significantly improving the resolution. Furthermore, as we demonstrated in our previous research biomolecules within the trap can be independently manipulated in two dimensions.[2] Photoelectrophoretic traps provide an additional level of control on the separation process. Implementation of photoelectrophoretic traps in microfabricated devices will further enhance the level of control on the dynamics of biomolecules and may lead to many innovative types of lab-on-a-chip devices.

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