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Optimization of ELFSE DNA Sequencing with EOF Counterflow and Microfluidics

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Abstract

We present a non-linear optimization study of different implementations of the DNA electrophoretic method "End-labeled Free-solution Electrophoresis" (ELFSE) in commercial capillary electrophoresis systems and microfluidics to improve the time required for readout. Here, the effect of electro-osmotic counterflows (EOF) and snap-shot detection are considered to allow for detection of peaks soon after they are electrophoretically resolved. Using drag tags available in micelle form, we identify a design capable of sequencing 600 bases in 2.8 minutes.

Introduction

In conventional micelle end-labeled free solution electrophoresis, electro-osmotic flow is suppressed and a finish-line detector is used to observe long DNA migrate first with short DNA to eventually follow. This detection mode is considered conventional as it is readily available through the use of commercial capillary electrophoresis devices. The conventional detection mode is ideal for gel electrophoresis which is the current standard method for DNA separation. This detection mode is not necessarily ideal, however, for end-labeled free solution electrophoresis.

End-labeled free solution electrophoresis (ELFSE) is rapid gel-free DNA separation technique. DNA has a length independent electrophoretic mobility in gel-free solution. This arises as the number of charges and the amount of hydrodynamic friction imparted on to the DNA both scale linearly with DNA length. The scaling can be broken by attaching an uncharged drag tag to the DNA. The size and polydispersity of the drag tag has a significant impact on the quality of the ELFSE separation. Several drag tags have been investigated including natural linear proteins,¹ comblike polypeptoids,² genetically engineered proteins^{3,4} and non-ionic surfactant micelle drag tags.^{5,6} It was shown that micelles produce superior ELFSE separations due to their increased size while maintaining low polydispersity.

Long DNA, bearing more charge, will migrate quickly compared to short DNA which is significantly retarded by the drag tag. While long DNA migrates the fastest, it is also the most difficult to resolve due to small difference in the drag tag-DNA complex mobility.

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Once the long DNA is resolved, every other DNA length in the capillary is also resolved, but the separation is not complete until the short DNA passes the detector. For end-labeled free solution electrophoresis it is thus advantageous to detect all the DNA lengths of interest as soon as the length of read becomes resolved.

McCormick and Slater⁷ presented a theoretical study on how to use an electro-osmotic flow (EOF) to reverse the elution order of the end-labeled free solution electrophoresis separation. The study used physical properties from a previously published experimental study by Ren, et al.¹ In the theoretical study by McCormick and Slater, it was shown that long DNA, bearing more charge than short DNA, can better resist an EOF counter-flow and will stay in the capillary longer giving it more time to separate. Short DNA, which is easily separated, elutes out of the capillary quickly. Unfortunately using an EOF counter-flow opens the possibility of having DNA either eluting too quickly (under separated) or not eluting at all when EOF balances with electrophoresis. McCormick and Slater showed that a range of EOF counter-flows will significantly extend the read frame of ELFSE separations. This read frame extension comes at the expense of run time, however which requires some consideration to examine the tradeoff. These tradeoffs can be examined explicitly though the use of mathematical optimization.

In addition, we also investigate the use of snap-shot detection to render a fast detection method for ELFSE. Pfeiffer et al.⁸ showed that non-linear optimization can be used to find the smallest microfluidic device to complete a generic separation using electrophoresis. We present a similar non-linear optimization strategy that instead minimizes the run time of the separation and find the necessary size of the microfluidic device to do so. Throughout, we will choose drag-tag size, wall adsorption, and Joule heating parameters that are typical for micelle-ELFSE methods, but the results are generalizable to any ELFSE method that can provide fairly large drag-tags with good monodispersity and wall adsorption characteristics.

Controlled EOF counter-flow

To render a model of the EOF-ELFSE process, we will now consider the physics in more detail. The negative charge laden glass capillary wall is balanced by positive counter-ions within the double layer while the bulk is electro-neutral. When an electric field is applied the positive counter-ions will slip toward the cathode pulling the bulk fluid in an electro-osmotic flow. DNA is negatively charged and will undergo electrophoresis in the opposite direction. The net electrokinetic mobility of the drag-tag DNA complex is

$$\mu = \mu_{EOF} - \mu_0 \left(\frac{L}{L+\alpha} \right) \quad (1)$$

where μ_{EOF} is the electro-osmotic mobility, μ_0 is the free solution electrophoretic mobility of DNA, L is the number of DNA bases, and α is the size of drag tag. The elution time is then calculated by $t = l_D/\mu E$ or

$$t = \frac{l_D}{E} \left(\frac{L+\alpha}{\mu_{EOF}(L+\alpha) - \mu_0 L} \right) \quad (2)$$

where l_D is the capillary length to the detector, E is the applied electric field strength, and μ_{EOF} is the EOF mobility. If EOF mobility is greater than the free solution electrophoretic mobility of DNA ($\mu_{EOF} > \mu_0$) then EOF dominates and DNA will elute from shortest to longest as short DNA bears little charge, and therefore, low resistance to EOF, and is pushed out of the capillary first. This is commonly the situation when working with bare-silica capillaries which have typical EOF mobilities of $\mu_{bare} = 3.6 \times 10^{-4} \text{ cm}^2/\text{V}\cdot\text{s}$ compared to a typical free solution DNA mobility of $2.4 \times 10^{-4} \text{ cm}^2/\text{V}\cdot\text{s}$.⁹ Unfortunately bare-silica capillaries impose such a large EOF that the DNA is poorly separated when it elutes passed the detector. The separation of DNA lengths in CE is given by the temporal spacing

$$\left| \frac{\partial t}{\partial L} \right| = \frac{l_D}{E} \frac{\alpha \mu_0}{(\mu_{EOF}(L+\alpha) - \mu_0 L)^2} \quad (3)$$

which is a strong function of the drag tag size and EOF. The proper choice of EOF mobility and drag tag size will yield infinite separation for DNA length L but the elution time will also go to infinity as this particular DNA length has zero net mobility. Using EOF, the temporal spacing can be optimized to suit the separation of long DNA.

Temporal spacing alone is not sufficient to quantify the quality of the separation. In CE, DNA is injected into the capillary as a square plug which, over the course of the separation, evolves to a Gaussian profile due to diffusion and other velocity perturbing effects such as wall adsorption and drag tag polydispersity. If the peaks are broad compared to the spacing then the profiles will be poorly resolved as indicated by the resolution factor, R_L

$$R_L = \frac{\sqrt{8 \ln(2) \sigma_t^2}}{|\partial t / \partial L|} \quad (4)$$

where σ_t^2 is the variance of the Gaussian profile so that the resolution factor is the ratio of the full-width at half maximum of the peak to the temporal spacing of peaks whose oligomers differ by a single base in length. We have found that the condition $R_L \geq 1.5$ is required to confidently identify neighboring oligomers differing by a single base in length. The temporal variance is related to the spatial variance through the velocity

$$\sigma_t^2 = \frac{\sigma_x^2}{(\mu E)^2} \quad (5)$$

where μ is given by Eq. (1). The significant contributions to the variance are given by diffusion, wall adsorption, and Joule heating^{1,4}

$$\sigma_x^2 = 2Dt + W\mu E l_D + \frac{J\mu E^5 l_D}{D} \quad (6)$$

where D is the DNA diffusivity, which scales with DNA length according to

$D = D_1 / \sqrt{L + \alpha}$, with $D_1 = 4.43 \times 10^{-6} \text{ cm}^2/\text{s}$,⁷ J is the Joule heating parameter with $J = 2.72 \times 10^{-12} \text{ cm}^2/\text{kV}^4$, and W is the wall adsorption parameter with $W = 16.7 \text{ }\mu\text{s}$.^{9,10} The

drag tag sizes are allowed to vary between 0 and 502, where $\alpha = 502$ is a drag tag size rendered from a mixed micelle solution of $C_{12}E_6$ surfactant.⁹

Now that we have defined the variance generation and the peak spacing above, we can identify the optimal EOF mobility, drag tag size, capillary length, and electric field strength by solving the optimization problem

$$\min t_{run} = \frac{l_D}{\mu_0 E} \left(1 + \frac{\alpha}{L} \right)$$

$$\text{s.t. } R_L \leq 1.5, L = L_{short}, L_{short} + 1, \dots, L_{LOR}$$

$$R_L = \frac{2 \sqrt{2 \ln(2) \sigma_t^2}}{|\partial t / \partial L|}$$

$$\left| \frac{\partial t}{\partial L} \right| = \frac{l_D}{E} \frac{\alpha \mu_0}{(\mu_{EOF}(L + \alpha) - \mu_0 L)^2}$$

$$E = V_{app} / l_C$$

$$l_C = l_D + \delta l$$

$$\sigma_t^2 = \frac{\sigma_x^2}{(\mu E)^2}$$

$$\sigma_x^2 = 2Dt + W \mu E l_D + \frac{J \mu E^5 l_D}{D} \quad (7)$$

$$D = \frac{D_1}{\sqrt{L + \alpha}}$$

$$t = \frac{l_D}{\mu E}$$

$$\mu = \mu_{EOF} - \mu_0 \left(\frac{L}{L + \alpha} \right)$$

$$\mu \geq 0$$

$$0 \leq \mu_{EOF} \leq \mu_{bare}$$

$$0 \leq \alpha \leq \alpha_{max}$$

$$l_{min} \leq l_D \leq l_{max}$$

$$0 \leq V_{app} \leq V_{max}$$

where L_{short} is the shortest DNA length of interest, typically the primer which we take to be 26 bases long, and L_{LOR} is the length of read which is the longest DNA length to have single base resolution.

Note that the run time is set by the length of read as the constraint $\mu \geq 0$ ensures EOF dominates the flow throughout the design space which leads to a reversal of the elution order of the DNA. The optimization problem can also be reformulated for the electrophoresis dominant case by replacing $\mu \rightarrow -\mu$ and $E \rightarrow -E$ indicating that the net mobility now causes DNA to move in the opposite direction but still heads to the detector as the polarity of the electric field is flipped. Optimization shows that the electrophoresis dominant case should be run completely EOF suppressed and is therefore identical to the conventional method discussed previously.¹⁰

In the results section, we will see that partially suppressed EOF active capillaries can be used to significantly speed up the DNA separation. Partial suppression of EOF can be difficult to control, however. We will also see that the optimal EOF mobility strongly depends on the free solution electrophoretic mobility of DNA μ_0 which may vary between experiments. We take the bare-silica EOF mobility to be $\mu_{bare} = 3.6 \times 10^{-4} \text{ cm}^2/\text{V}\cdot\text{s}$.

The results shown in the next section are generated by solving the optimization problem (7). The constraints listed in the optimization problem require that at least single-base resolution is attained for every DNA length of interest according to the mathematical model. We vary the length of read from 30 to 600 bases in intervals of 10 bases to generate smooth lines on each figure. The optimization problems are solved in MATLAB v 8.1.0.604 (R2013a) using the `fmincon` built in function with the options set to use the `sqp` algorithm. This algorithm solves a quadratic approximation of the optimization problem at a user-supplied initial

guess. Once the initial quadratic problem is solved, a new quadratic approximation is made at the candidate solution and the new quadratic problem is solved to generate a better candidate solution. The successive quadratic program is repeated until convergence is attained. Quadratic optimization problems are convenient to solve since their derivatives are linear. This allows for the direct solution of the quadratic optimization problem using linear algebra.

EOF-ELFSE Optimization results

The minimum run times of the partially suppressed EOF active ELFSE DNA separation is shown in fig 2. The optimization shows that a single partially EOF suppressed capillary in an ABI 3130x1 can separate 600 bases in 4.35 minutes. Fig 3 shows the optimal EOF mobility and the optimal run time for a length of read of 600 bases is a strong function of the free solution electrophoretic mobility of DNA.

Snap-shot detection

In this section, we will discuss the modeling and optimization framework for using snap-shot detection mode in micelle end-labeled free solution electrophoresis DNA separations. Snap-shot detection is the instantaneous imaging of the entire separation channel. Snap-shot detection is capable of imaging the entire DNA separation the moment the length of read is resolved. Snap-shot detection is not without its own challenges however. Instantaneous imaging of an entire separation channel (typically between 5 and 20 cm) can be performed using a microfluidic device on a motorized stage. The size of microfluidic devices varies considerably. Turns are often required in order to fit a sufficiently long channels on to the microfluidic device. Figure 4 shows the serpentine and spiral configurations which are commonly used to fit long separation channels on a small chip area.

When using snap-shot detection, there is no benefit in reversing the elution order of the DNA by using an EOF counter flow. DNA will migrate the fastest with EOF fully suppressed. Hence, the electrophoretic mobility, μ , of the micelle-DNA complex is

$$\mu = \mu_0 \frac{L}{L + \alpha} \quad (8)$$

where μ_0 is the free-solution mobility of DNA, L is the length of DNA in bases, and α is the size of the micelle in units of uncharged DNA bases that have the equivalent drag of the micelle. With snap-shot detection the DNA molecules are observed as they migrate down the separation channel. As the DNA molecules migrate down the separation channel, they separate into different concentration profiles that are Gaussian in shape, when averaged across the cross-section of the separation channel, as shown in Figure 5. The concentration profiles are resolved from each other when the average full-width at half-maximum, w_L , is less than 1.5 times the spacing between maximums, x_L ,

$$R_L = \frac{w_L + w_{L+1}}{2\Delta x_L} \leq 1.5 \quad (9)$$

where R_L is the resolution factor. The spacing between peaks is given by

$$\Delta x_L \approx \frac{\partial x}{\partial L} = \mu_0 E t \frac{\alpha}{(L+\alpha)^2} \quad (10)$$

where t is the time at which the Gaussians are detected. With snap-shot detection, the full-width at half-maximum is a measure of the Gaussians width as it evolves in space

$$w_L = 2 \sqrt{2 \ln(2) \sigma_x^2} \quad (11)$$

where σ_x^2 is the spatial variance for DNA of length L .

The convection-diffusion equation can be used to model the effect of the microfluidic device topology on the concentration profiles as the DNA lengths migrate through the microfluidic device

$$\frac{\partial c_L}{\partial t} + \mathbf{u} \cdot \nabla c_L = D \nabla^2 c_L \quad (12)$$

where c_L is the concentration profile of DNA of length L , D is the diffusivity, and $\mathbf{u}$ is the electrophoretic velocity vector. The convection-diffusion equation (12) can be solved analytically, using a few simplifying assumptions, to reveal the model for the variance generation σ_x^2 as the concentration profile propagates down the microfluidic channel.^{11–13}

The primary simplification to the convection diffusion equation (12) deals with the velocity of the DNA in a curved microfluidic channel. In a straight channel, the velocity is constant. In a microfluidic turns, the DNA velocity varies across the interior of the channel. This is because the outside channel has a longer contour length than the inside channel which also results in an electric field gradient. Defining the x coordinate to be along the axial direction of the microfluidic channel and the y coordinate to be pointing to the interior of the microfluidic channel (see figure 6), the velocity is given by $\mathbf{u} = u(y)\mathbf{e}_x$ where $\mathbf{e}_x$ is the unit vector pointing in the axial direction^{11–13}.

The convection-diffusion equation (12) is two-dimensional in (x, y) and is solved analytically to determine σ_x^2 as a function of the microfluidic structure^{12,13},

$$\sigma_x^2 = \sigma_0^2 + 2Dt + \sum_{i \in I} \sigma_{skew,i}^2 + \sum_{j \in J} \sigma_{turn,j}^2 + \sigma_{other}^2 \quad (13)$$

where σ_0^2 is the initial variance of the injected Gaussian profile, $2Dt$ is the variance caused by diffusion over the migration time t , DNA diffusivity scales as $D = D_1 / \sqrt{L+\alpha}$ where D_1 is constant⁷, $\sigma_{skew,i}^2$ is the variance caused by a skewed concentration profile as it enters section i , I is the set of all sections in the microfluidic device, $\sigma_{turn,j}^2$ is the variance caused by the concentration profile migrating through each turn, J is the set of all turn sections in the microfluidic device, and σ_{other}^2 is any other source of variance not modeled by the convection-diffusion equation. Here a section is defined as either a straight channel or a semi-circular turn. A spiral consists only of semi-circular turn sections while a serpentine

Figure 6 shows the variance generated by turns in a microfluidic device. After a turn, the concentration band becomes skewed. The skewed concentration band causes the variance to increase as the concentration gradient cause diffusion to be exacerbated. The variance increase due to concentration profile skew is

$$\sigma_{skew,i}^2 = \sum_{n=1,3,5,\dots}^{\infty} S_n^{(i)} \Gamma_n^{(i)} \quad (14)$$

where $\Gamma_n^{(i)} = \pm \frac{8\mu E w_c^2}{r_i D (n\pi)^4} \left(1 - e^{-(n\pi)^2 D t_i / w_c^2}\right)$, μ is the mobility given by Eq. (8), E is the applied electric field, w_c is the channel width, r_i is the radius of the center-line of the turn, D is the diffusivity, t_i is the time it takes to get through the turn or straight section i , i.e. $t_i = l_i / (\mu E)$ where l_i is length of the turn or straight channel. The term $S_n^{(i)}$ indicates the concentration band skewness as it enters section i .

$$S_n^{(i)} = \begin{cases} S_n^{(i-1)} e^{-(n\pi)^2 D t_i / w_c^2}, & (i-1) \in K \\ S_n^{(i-1)} e^{-(n\pi)^2 D t_i / w_c^2} + \Gamma_n^{(i-1)}, & (i-1) \in J \end{cases} \quad (15)$$

where K is the set of straight sections, J is the set of turn sections, and the initial concentration profile is assumed to be unskewed, $S_n^{(1)} = 0$. The sign on the terms $\sigma_{skew,i}^2$ and $\Gamma_n^{(i)}$ is indicated by the orientation of the turns with respect to the y -axis. If the center of the turn is pointing away from the positive y -direction then the sign on $\sigma_{skew,i}^2$ and $\Gamma_n^{(i)}$ is positive, if the center of the turn is pointing toward the positive y -direction then the sign on $\sigma_{skew,i}^2$ and $\Gamma_n^{(i)}$ is negative. In figure 6 the sign of $\sigma_{skew,i}^2$ and $\Gamma_n^{(i)}$ on the right turn is positive and the sign on the left turn is negative. The sign changes with each successive turn which indicates how the complementary turns can be used in a serpentine to limit the development of peak broadness. The sign of $\sigma_{skew,i}^2$ and $\Gamma_n^{(i)}$ is always positive for a straight channel (after a turn) and a spiral. The spiral configuration does not allow for complementary turns and instead uses large turn radii to mitigate against large variance development. As the turn radius tends to large values, the turn can be well approximated as a straight channel as variance generation due to the race track effect becomes negligible.

Turns in microfluidic devices introduce variance into the concentration profile due to the non-uniform velocity across the width of the channel. This variance generation is quantified by

$$\sigma_{turn,j}^2 = \left(\frac{8\mu E w_c^3}{r_j D} \right)^2 \sum_{n=1,3,5,\dots}^{\infty} \frac{\Phi_n(t_j)}{(n\pi)^8} \quad (16)$$

where $\Phi_n(t_j) = -1 + e^{-(n\pi)^2 D t_j / w_c^2} + (n\pi)^2 D t_j / w_c^2$.

The term σ_{other}^2 in Eq. (13) refers to any other sources of variance not modeled by the convection-diffusion equation (12). As presented above, the other significant sources of variance are wall adsorption and Joule heating rendering the complete variance

$$\sigma_x^2 = \sigma_0^2 + 2Dt + \sum_{i \in I} \sigma_{skew,i}^2 + \sum_{j \in J} \sigma_{turn,j}^2 + W\mu El_D + \frac{J\mu E^5 l_D}{D}. \quad (17)$$

The run time for micelle ELFSE in a microfluidic device is set by the instant the longest DNA length of interest (the length of read) is resolved. At that instant, the entire microfluidic device is scanned by the snap-shot detection and every DNA length is detected. The minimum snap-shot run time can be found by solving the following optimization problem

$$\min t_{run} = \frac{l_D}{\mu_0 E} \left(1 + \frac{\alpha}{L} \right)$$

$$\text{s.t. Eq.(8) - Eq.(11),}$$

$$\text{Eq.(14) - Eq.(17)}$$

$$D = D_1 / \sqrt{L + \alpha}$$

$$E = V_{app} / l_D$$

$$l_D = \sum_{i \in I} l_i \quad (18)$$

$$0 \leq \alpha \leq \alpha_{\max}$$

$$0 \leq V_{app} \leq V_{\max}$$

$$g(l_i, r_j) \leq X_{\max}$$

$$h(r_j) \leq Y_{\max}$$

$$c(l_i, r_j) \leq 0$$

where L is the length of read. The constraints g , h , and c are geometric constraints that ensures that the microfluidic device fits the specified area and ensures that all the sections are contiguous, $g(l_i, r_j) = h(r_j) = 2r_j$, $\forall j \in J$ for a spiral or $g(l_i, r_i) = l_i + r_{i+1} + r_{i-1}$, $\forall i \in I$ and $h(r_j) = \sum_{j \in J} 2r_j$ for a serpentine, $X_{\max}$ and $Y_{\max}$ is the maximum length allowed for the horizontal and vertical side of the microfluidic device, respectively. A serpentine is assumed to have straight channels aligned in the horizontal direction. In addition, spirals have the constraint $c(l_i, r_j) = \delta r - (r_{j-1} - r_j)$, $\forall j \in J$, which prevents the turns from overlapping. For serpentes the constraint $c(l_i, r_j) = l_i - l_{i-1}$, $\forall i \in I$ guarantees connectivity between the turns and each straight channel.

The parameters are specified to match DNA in a micelle solution, i.e. $\mu_0 = 2.4 \times 10^{-4}$ cm²/V·s, $D_1 = 4.43 \times 10^{-6}$ cm²/s, $\alpha_{\max} = 502$ and $W = 16.7$ μ s. The maximum dimensions of the device are set at $X_{\max} = Y_{\max} = 10$ cm. The minimum gap between turns in a spiral δr is set at 0.1 cm, which allows for practical fabrication of the device¹⁴. The maximum applied voltage is $V_{\max} = 20$ kV.

Snap-shot detection optimization results and discussion

The minimum run times of the snap-shot detection modes are shown in fig 7. For convenience we also show the results for single and parallel capillaries in an ABI 3130x1, and the EOF-ELFSE results from the previous section.

From the optimization results it is clear that, while all are fast, snap-shot detection implemented using microfluidics offers the fastest run time. The optimal microfluidic structure for a length of read of 600 bases is the serpentine, where optimization finds the required channel length to be 20.8 cm. Fig 8 shows the minimum run time for achieving a length of read of 600 bases in both a serpentine and a spiral. For serpentes the run time decreases as the number of sections increases although the total optimal channel length is identical for serpentes with more than five sections. This requires tighter turn radii which can be mitigated by design of the complementary turns. That is, the serpentine is designed such that the skewed concentration band exiting a turn migrates at a high enough velocity to avoid significant dispersion before the next complementary turn. For spirals the run time increases with increasing number of sections. The design constraints are such that the spiral must increase the channel length every time an additional turn is used even though the increased channel length comes at the expense of run time.

Conclusions and Future Work

In this paper we showed that alternate detection modes, such as a controlled EOF counter-flow and snap-shot detection can be used to significantly reduce the run time for micelle end-labeled free solution electrophoresis DNA separations. Microfluidic serpentes are shown to be the optimal configuration for micelle ELFSE which can separate 600 bases in 2.76 min using five sections. Spirals can be used to resolve a length of read of 600 bases in

3.50 min using two turn section. The partially EOF suppressed capillary is the slowest of the these alternate modes, resolving 600 bases in 4.35 min in an ABI 3130xl.

This theoretical study outlines the top performance we expect from a microfluidic device and an EOF active DNA separation using micelle end-labeled free solution electrophoresis. There are a few assumptions we made that may be difficult to realize in practice. Chief among them is the lack of delay in snap-shot detection. We assumed that the separation can be imaged the instant the length of read becomes resolved, when in actually there will be some delay due to the detector scanning the separation channel. This delay is equipment dependent and may vary significantly from lab to lab. Other limitations of applying this theoretical study to experiments include the use partially EOF suppressed capillaries which are typically available in a few EOF suppression levels. The partial EOF suppression may be prone to drift as the capillary degrades leading to failure of the DNA separation. The theoretical models we used this work have been used by previous researchers and show excellent agreement with experimental data. As with any theoretical study, however, there is a possibility that some unmodeled physics will be significant in the experimental validation of these results. For instance, the effect of the channel depth in the microfluidic device is unmodeled and we expect the typical trade-off in signal-to-noise ratio and fabrication costs to drive the selection of channel depth. Once all the essential physics have been modeled and parameters properly tuned to fit experimental data, the general approach for finding the optimal DNA separation device which we present in the work will remain unchanged.

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References

1. Ren H, Karger A, Oaks F, Menchen S, Slater G, Drouin G. Electrophoresis. 1999; 20:2501–2509. [PubMed: 10499343]
2. Haynes R, Meagher R, Won J, Bogdan F, Barron A. Bioconjugate Chemistry. 2005; 16:926–938.
3. Meagher R, Won J, Coyne J, Li J, Barron A. Analytical Chemistry. 2008; 80:2842–2848. [PubMed: 18318549]
4. Albrecht JC, Lin JS, Barron AE. Analytical Chemistry. 2011; 80:509–515. [PubMed: 21182303]
5. Grosser ST, Savard JM, Schneider JW. Analytical Chemistry. 2007; 79:9513–9519. [PubMed: 18020426]
6. Savard JM, Grosser ST, Schneider JW. Electrophoresis. 2008; 29:2779–2789. [PubMed: 18546164]
7. McCormick LC, Slater GW. Electrophoresis. 2006; 27:1693–1701. [PubMed: 16568501]
8. Pfeiffer AJ, Mukherjee T, Hauan S. Industrial & Engineering Chemistry Research. 2004; 43:3539–3553.
9. Istivan, SB. Ph.D. thesis. Pittsburgh, PA: Carnegie Mellon University; 2012. Gel-free Separations of DNA via Transient Attachment to Surfactant Micelles; p. 5213
10. Fahrenkopf MA, Ydstie BE, Mukherjee T, Schneider JW. Computers & Chemical Engineering. 2014; 64:63–70. [PubMed: 24764606]
11. Griffiths SK, Nilson RH. Analytical chemistry. 2000; 72:5473–5482. [PubMed: 11080903]
12. Molho J, Herr A, Mosier B, Santiago J, Kenny T, Brennen R, Gordon G, Mohammadi B. Analytical Chemistry. 2001; 73:1350–1360.
13. Wang Y, Lin Q, Mukherjee T. Lab on a Chip. 2004; 4:453–463. [PubMed: 15472729]

14. Culbertson CT, Jacobson SC, Ramsey JM. Analytical Chemistry. 2000; 72:5814–5819. [PubMed: 11128941]

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Figure 1.

Diagram of an EOF active micelle ELFSE separation. EP labels electrophoresis and EOF labels electro-osmotic flow.

Figure 2. Optimal run time for partially EOF suppressed capillaries (—). Optimal values for drag tag size and the applied voltage are the maximum available ($\alpha_{\max} = 502$ and $V_{\max} = 20$ kV). The free-solution electrophoretic mobility is taken to be $\mu_0 = 2.4 \times 10^{-4}$ cm²/V · s.

Figure 3. Sensitivity of optimal EOF mobility and optimal run time to free solution mobility for 600 bases of length of read. The curve for EOF mobility is indicated by an arrow pointing left toward its own y-axis. The curve for run time is indicated by an arrow pointing right toward its own y-axis.

Figure 4. (A) Microfluidic spiral, (B) microfluidic serpentine. The different configurations are composed of either straight sections or semi-circular turn sections which are indicated by dashed line.

Figure 5.
Band broadening in snap-shot detection.

Figure 6.

Concentration profiles are broadened by turns. The variance of the concentration profile is increased during each turn and quantified by $\sigma_{turn,j}^2$. The initial concentration profile is unskewed. After the first turn the concentration profile becomes skewed and diffusion is enhanced by the concentration gradient which results in variance generation $\sigma_{skew,3}^2$. The second turn is complementary which subtracts some variance $\sigma_{skew,4}^2$. The index of each section i is specified at the section exit.

Figure 7.

Optimal run time for (1) single capillary, (2) two parallel capillaries, (3) three parallel capillaries, (4) partially suppressed EOF counter-flow, (5) spiral microfluidic device, and (6) serpentine microfluidic. The optimal range of drag tag size varies from 14 to 502 as the length of read increase. The optimal drag tag size of 502 is found for lengths of read above 450 bases. The optimal voltage ranges from 10 kV to 20 kV where both long and short length of read are optimal with large voltage and lengths of read between 200 and 420 require voltages around 10 kV.

Figure 8. Optimal run time for a length of read of 600 bases as a function of the number of sections in a microfluidic serpentine (—●—) and a microfluidic spiral (—■—).