Optimization of sample transfer in two-dimensional microfluidic separation systems†

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Multidimensional microfluidic separation systems combining a first dimension microchannel with an array of parallel second dimension microchannels can suffer from non-uniform sample transfer between the dimensions, sample leakage, and injection plug tailing within the second dimension array. These factors can significantly reduce overall two-dimensional separation performance. In this paper, numerical and analytical models reveal an optimized chip design which combines multidimensional backbiasing and an angled channel geometry to ensure leakage-free and uniform interdimensional sample transfer, while also minimizing injected sample plug lengths. The optimized design is validated experimentally using a multidimensional chip containing five second dimension channels.

Introduction

There has been considerable progress in the development of microfluidic systems for biomolecular separations based on a variety of single dimension electrokinetic separation mechanisms.5–7 For the analysis of complex biological samples, single dimension separations typically cannot provide sufficient resolving power for effective analysis of these complex mixtures. Thus, microfluidic systems employing multidimensional separations such as isoelectric focusing (IEF)-sodium dodecyl sulfate (SDS)/capillary gel electrophoresis (CGE),5–7 IEF-capillary zone electrophoresis (CZE),8 and CZE-micellar electrokinetic chromatography (MEKC),9 have been developed for achieving higher peak capacity than single dimension systems, while leveraging the inherent advantages of microfluidics to seamlessly couple the multiple separation dimensions on-chip.

Microfluidic platforms for multidimensional separations can be either time-multiplexed, with second dimension separations performed serially,8,10,11 or spatially-multiplexed, with multiple parallel second dimension separations performed simultaneously.7,12,13 In time-multiplexing, sample fractions from the first separation dimension are sequentially sampled and separated within the second dimension. To enable reasonable sampling of analyte from the first dimension while limiting sample loss and band broadening between sequential sampling steps, the second dimension separation must be substantially faster than the first. This constraint limits the available separation modes which may be employed in time-multiplexed systems.

In contrast, spatially-multiplexed separations relax this requirement by allowing the first separation dimension to be fully sampled in a single step, thus enabling access to a wider range of high-resolution separation modes.14 Spatially-multiplexed microfluidic systems for intact protein separations based on IEF-CGE has been an area of particular focus in recent years.5,7,13–15 While the peak capacity of chip-based IEF-CGE systems remains lower than traditional slab gel two-dimensional (2-D) polyacrylamide gel electrophoresis (PAGE), microfluidics technology has been used to reduce the overall separation time by 2 orders of magnitude,7,14 with substantially lower sample loading requirements and improved separation repeatability comparable to slab gels.14

In spatial-multiplexing, efficient transfer of sample between the dimensions plays a critical role in achieving good separation performance. Repeatability and uniformity of sample plug injection into each of the parallel second dimension channels are important metrics. For example, if the injection dynamics differ between the second dimension channels, a large sample band which co-elutes into adjacent channels will exhibit different migration times at the downstream detector and appear as two or more separate bands, rather than a single band spread over multiple channels. Avoiding dispersion of analyte plugs during the injection process is another important concern. If the initial injected sample plug length is larger than the characteristic analyte diffusion length during the second dimension separation, the system will not be capable of reaching its optimal diffusion-limited separation efficiency.14

The electrokinetic injection of defined sample plugs within single dimension microfluidic systems has been extensively studied, with the most widely used injector configurations including the cross,16 double-T,17 and triple-T18 topologies, and extended configurations employing continuous sample-injection19 leveraging flow switching techniques.20 Floating-injection and pinched-injection methods have been employed in simple cross-injectors for the controlled definition of small sample plugs,21 while double- and triple-T designs are generally employed for the introduction of larger sample volumes.18 Regardless of the injector topology and injection method,

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sample leakage is a central issue which can dictate the effective injection plug length. After the desired sample plug has been transferred to the separation channel, additional samples can enter the injection region due to diffusion and fringing of the electrical field during the electrokinetic transfer process. This excess sample results in tailing of the injected sample plug which degrades separation performance. Backbiasing is a commonly-used method for eliminating sample leakage. In this approach, bias voltages are applied at the sample inlet and waste reservoirs in order to electrokinetically pull back excess sample from the injection zone. When suitable bias voltages are selected, backbiasing is effective at eliminating sample leakage for single-channel separation devices.

Just as with single dimension separation systems, efficient injection of sample plugs is a critical consideration in multidimensional systems when transferring analyte from the first to the second dimensions. While several groups have proposed 2-D chip designs consisting of an array of second dimension separation channels aligned with an identical number of injection channels on the opposite side of the first dimension microchannel,[12,13] a more efficient design for spatially-multiplexed 2-D separation chips is shown in Fig. 1(a). The design consists of a single first dimension separation channel intersected by \( n \) second dimension separation channels on one side, and \( n+1 \) sample injection channels on the opposite side, with the injection channels staggered with respect to the separation channels to ensure complete and simultaneous sampling of the first dimension channel.[14] Additional channels for multidimensional backbiasing, introduced in this paper, are also shown in the figure proximal to the sample inlet and sample waste reservoirs (dashed lines). In this staggered design, the parallel second dimension microchannels may be regarded as an array of double-T injectors operating in parallel. However, unlike the single-channel case, the double-T injectors are not electrically isolated in the multidimensional system. This interconnected design can result in significant variations in performance between the different injectors, as depicted in Fig. 1(b). In this image, sample initially within the first dimension channel is being electrokinetically transferred into the second dimension channel array by applying a uniform bias voltage in the injection channel reservoirs while grounding the second dimension reservoirs. Three main features are evident in this injection process. First, substantial tailing of sample occurs at the head of each second dimension channel, resulting in sample dispersion during transfer. Second, in all but the center second dimension channel, the injection is highly asymmetric, reflecting a non-uniform electric field distribution within the first dimension channel. Third, sample from the outermost regions of the first dimension channel continually leaks into the second dimension array due to a combination of diffusion and electric field fringing, leading to additional tailing which can continue long after sample from the center region of the first dimension channel has been fully transferred.

In the following, the causes of these performance issues are identified and evaluated through a combination of analytical modeling, numerical simulations, and experimental validation. Based on the results, a new chip design is proposed which employs multidimensional backbiasing channels, as depicted in Fig. 1(a), and a modified geometry for the first dimension microchannel. The resulting design eliminates injection asymmetry and sample leakage, while minimizing sample tailing issues which have previously limited performance of the staggered 2-D chip design.

**Methods**

**Reagents**

To minimize diffusion during experimental validation, bovine serum albumin (BSA) prelabeled by Alexa Fluor-555 (Invitrogen; Calsbad, CA, USA) was used as a low-diffusivity analyte. Acrylamide (AAm), \( N,N' \)-methylenbisacrylamide (Bis), ammonium persulfate (APS), \( N,N',N' \)-tetramethylenediamine (TEMED), 3-(trimethoxysilyl)propyl methacrylate (TPM) and poly(vinyl alcohol) (PVA) were purchased from Sigma–Aldrich Inc. (St. Louis, MO, USA). Sodium dodecyl sulfate (SDS), urea, dithiothreitol (DTT), tris(hydroxymethyl)-aminomethane (Tris), methanol, isopropyl alcohol (IPA), and concentrated hydrochloric acid (HCl) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). HPLC-grade DI water was used for sample and prepolymer solution preparation.

**Sample preparation**

Sample buffer was prepared by adding 0.25 mL 0.5 M Tris-HCl (pH 6.8), 0.4 mL 10% SDS in DI water, 0.2 mL glycerol, and 0.031 g DTT in sequence. DI water was added for final concentrations of 4% SDS, 20% glycerol, 0.2 M DTT and 0.125 M Tris-HCl. Lyophilized BSA was added to the buffer.
solution at 1 mg mL$^{-1}$ in a tube, and denatured by placing the tube in a water bath at 100 °C for 90 s. Prior to use, the final sample was diluted to 10 μg mL$^{-1}$ with DI water.

**Chip fabrication**

Microchannels were fabricated in a 1.5 mm thick sheet of polymethylmethacrylate (PMMA), using a standard PMMA containing UV stabilizers (FF grade; Cyro; West Paterson, NJ, USA). Reservoirs were formed in a cover plate fabricated from a UV-transparent grade of PMMA (UVT grade; Spartech; Clayton, MO, USA). The channels were directly patterned by computer numerical control (CNC) milling (MDX-650A; Roland ADS; Lake Forest, CA, USA) using a 100 μm diameter end mill. The nominal geometry of each channel is 100 μm wide and 100 μm deep. The reservoirs were drilled by the same CNC milling machine, using a tool diameter of 1.8 mm. After machining, both PMMA wafers are cleaned by methanol, IPA and DI water sequentially in a class 1000 cleanroom environment, followed by aggressive drying with an N2 gun.

To ensure uniform and repeatable experimental results, it was necessary to minimize both diffusion and hydrodynamic flow within the polymer chips. To this end, ~1 cm long plugs of polyacrylamide gel were formed within the injection channels and second dimension channels. The wafers were first oxidized using a UV-ozone system (Novascan Technologies; Ames, IA, USA) for 8 min. After oxidation, the PMMA wafers were immediately immersed in a silanization solution consisting of $6:1:1000$, TPM: HC1: DI water v/v/v for 1 h, following the method described by Zangmeister and Tarlov.22 After drying, the TPM-treated wafers were thermally bonded in a hot press (Auto Four; Carver Inc.; Wabash, IN, USA) at 85 °C and 3.45 MPa for 15 min. The bonded chip was next filled with a solution of 4% (wt) acrylamide in DI water with 0.1% SDS mixed with 10% APS in DI water and TEMED 100 : 1 : 11 v/v/v. The solution was sonicated for 1 min prior to injection into microchannels. After 5 min polymerization time, the prepolymer remaining in the first dimension channel was vacuumed and replaced with 4% acrylamide + bis prepolymer, which diffuses into the APS/TEMED-containing prepolymer within the injection and second dimension channels to form a crosslinked polyacrylamide interface following a 2 h polymerization step. Because the prepolymer solution within the first dimension channel does not contain APS/TEMED, no photopolymerization occurs within this channel, while linear polyacrylamide (LPA) forms within the injection and second dimension channels beyond the diffusion length ~1 cm from the first dimension channel. The resulting gels provide a well-defined hydrodynamic barrier to limit sample dispersion out of the first dimension channel due to unavoidable pressure gradients. Finally, BSA sample at a concentration of 10 μg mL$^{-1}$ was introduced into the first dimension channel by capillary action immediately prior to testing.

**Detection**

Optical detection was performed using an inverted fluorescence microscope (Nikon Eclipse TE2000s; Nikon Inc.; Melville, NY, USA) with a 4× objective and a low-noise CCD camera (CoolSnap HQ2; Roper Scientific; Tucson, AZ, USA) with a frame rate of 10 fps.

**Results and discussion**

**First dimension current variations**

Consider the interdimensional injection region of a five-channel staggered chip design depicted in Fig. 2. Sample transfer is performed by applying equal bias voltages at each of the lower separation channel reservoirs, with all upper injection channel reservoirs grounded. Upon biasing, the $i^{th}$ injection channel exhibits an average current $I_i$, and the $j^{th}$ second dimension separation channel exhibits an average current $I_{j2}$. The average current within the segment of the first dimension microchannel between the $i^{th}$ injection channel and $j^{th}$ separation channel is defined as $I_{ij}$, with positive orientations defined in Fig. 2. Using PSpice circuit analysis software (Cadence Design Systems Inc., San Jose, CA, USA), the average currents can be readily determined. Using lengths of $L_i = 1 \text{ cm for each injection channel, } L_j = 400 \mu \text{m for each first dimension microchannel segment and } L_5 = 5 \text{ cm for the second dimension separation channels, and equal cross-sectional dimensions for all channels, normalized current variations within the ten segments of the first dimension channel are given in Fig. 3. In this simulation, equal resistivity was assumed within the injection and second dimension channels, while 4× lower resistivity was assumed within the first dimension channel to account for typical conductivity differences resulting from the high sample concentration within this channel before transfer.
seen by considering a chip containing three injection channels and two second dimension separation channels. Following the nomenclature defined in Fig. 2, the first dimension channel comprises 4 segments with currents $I_{1,1}$, $I_{1,2}$, $I_{1,3}$, and $I_{1,4}$. In the following analysis, the resistances of each injection channel, first dimension channel segment, and second dimension channel are denoted $R_{i}$, $R_{1}$, and $R_{2}$, respectively. Due to symmetry, only half of the circuit need be analyzed. Focusing on the first, second dimension channel and noting that the voltage drop from the injection reservoirs to the channel inlet must be equal for both the first and second injection channel, we have

$$I_{1}R_{i} + I_{1,1}R_{1} = I_{2}R_{i} + I_{2,1}R_{1}$$  \hspace{1cm} (1)

The current through each injection channel and its connected first dimension segment must also be equal, so that $I_{1,1} = I_{1}$ and $I_{2,1} = I_{2}/2$. The latter equality results from symmetry, since exactly half of $I_{1}$ is channeled towards the first, second dimension microchannel. Inserting both equalities into eqn (1) and solving for the ratio of currents in the 1st dimension segments results in

$$\frac{I_{1}}{I_{2}} = \frac{2R_{i} + R_{1}}{R_{1} + R_{2}}$$  \hspace{1cm} (2)

In general, the injection channels are substantially longer than the first dimension channel segments. With this assumption, $R_{i} \gg R_{1}$ and eqn (2) reduces to the simple equality $I_{1} = 2I_{2}$, i.e. a 100% variation in injection current for the case of a simple 2-D chip with only two second dimension channels.

To evaluate injection non-uniformity in chips containing greater numbers of second dimension channels, PSpice was used as a modeling tool. Simulation results for the case of a chip with five second dimension channels are shown in Fig. 4. In this figure, the ratios of current within each pair of first dimension segments connecting to a single second dimension channel is plotted against $R_{i}/R_{1}$, with the injection channel resistance held constant at $R_{i} = 50R_{1}$. The maximum current ratio occurs within the outermost pairs of first dimension segments. Furthermore, the current ratio increases with the second dimension resistance, approaching a value of 5 as $R_{i}$ increases towards infinity. Indeed, for any staggered chip design with $n$ second dimension channels and $n+1$ injection channels, the maximum current ratio will approach $n$ in the limit. This fact can be seen by considering that for $R_{i} >> R_{2} >> R_{1}$, the injection currents can be approximated as pure current sources applied to zero-resistance nodes between each second dimension channel. For the outermost first dimension segment, the full current from the outer injection channel ($I_{1}$) must pass through the first node, with a fraction $1/n$ entering the outer second dimension channel and $(n-1)/n$ continuing through the second node to feed the remaining second dimension channels. In the other direction, each of the remaining $n$ injection channels feeds $1/n$ of their total currents through the second node and into the outer second dimension channel, for a total current of $I_{2}$. Thus the maximum current ratio becomes

$$\lim_{R_{i} \to \infty} \left( \frac{I_{1}}{I_{2}} \right) = \frac{I_{1}}{I_{2}} \left( \frac{1}{1-(n-1)/n} \right) = n$$  \hspace{1cm} (3)

From eqn (3), injection uniformity clearly deteriorates as the density of the second dimension array is increased. However, higher density arrays are desirable to prevent undersampling of the first dimension separation and to provide increased peak capacity for the overall 2-D separation. Hence there is a need for methods to reduce or eliminate current asymmetries without constraining the number of second dimension channels.

**Multidimensional backbiasing**

One potential solution to the challenge of injection uniformity is to individually adjust voltages applied within each of the injection reservoirs shown in Fig. 1(a) to achieve equal currents within each first dimension channel segment. However, in addition to adding system complexity, this approach would require accurate and real-time knowledge of the resistivity within the various sections of the chip, and a feedback method for adjusting the voltages in response to any resistance variations resulting from changes in sample and buffer concentrations during the injection and separation processes.

A more practical solution is to modify the microchannel network to compensate for the variable network input resistance seen in an uncompensated, staggered injection design. Here we...
consider the use of backbias channels intersecting the outer regions of the first dimension channel, as shown in Fig. 1(a). These channels are named following the terminology from single dimension microfluidics, since the backbias channels serve a similar purpose in shunting current out of the ends of the sample transfer region during the injection process. Indeed, in addition to eliminating current non-uniformities during injection, the backbiasing channels also serve the same function as their single dimension counterparts by preventing sample leakage from the outer regions of the first dimension channel.

Consider the case where backbiasing channels are added to the simple design consisting of three injection channels and two second dimension channels. To simplify their integration, the backbias channels terminate at reservoirs biased at the same voltage as the second dimension microchannel reservoirs. Given backbiasing channels with equal resistances $R_b$ and average currents $I_b$, the value of $R_b$ which minimizes injection non-uniformity is found as follows. Noting that $R_1 << R_2$, and $R_1 << R_b$ and referring again to Fig. 2 and summing currents into the node connecting the first backbiasing channel with the first dimension channel yields

$$I_1 = I_{s1} + I_b \quad (4)$$

Inserting this into eqn (1), together with the previously-noted relationship $I_{s1} = I_1/2$, gives

$$I_{s1}(R_1 + R_b) + I_b R_b = I_{s1}(2R_1 + R_b) \quad (5)$$

For symmetric injection, the currents in both segments of the first dimension channel must be equal ($I_{s1} = I_{s2}$). In this case, solving eqn (5) for $I_b$ yields

$$I_b = I_{s1} = I_{s2} = I_1/2 \quad (6)$$

Also note that the voltage drop across the backbias channel is equal to the combined drop across the first, first dimension segment and the first, second dimension channel, i.e.

$$I_b R_b + I_b R_1 = I_{s1} R_1 + I_{s2} R_2 \quad (7)$$

Additionally, the current in the first, second dimension separation channel ($I_{s1}$) is simply the sum of the current through the first and second, first dimension segments, with the latter quantity equal to half the current from the second injection channel, that is $I_{s1} = I_{s1} + I_3/2$. Inserting this expression together with the equalities derived from eqn (6), eqn (7) reduces to:

$$R_b = 2R_1 \quad (8)$$

Thus a backbias channel with twice the nominal resistance of a second dimension separation channel will result in a perfectly balanced injection process, with equal currents within all first dimension channel segments.

Eqn (8) was derived for the case of a staggered injection chip with only two second dimension channels. To optimize backbiasing for chip designs containing larger numbers of second dimension channels, lumped-parameter numerical models were evaluated using PSpice. All analyses were performed with $R_1 = 0.2R_1$ and 4× lower resistivity within the first dimension channel to simulate a high concentration of analyte ions immediately following sample introduction. Designs containing up to nine second dimension channels were considered, with backbiasing resistances varying from $0.1R_1$ to infinity (i.e. no backbiasing). As a model case, the injection, first dimension, and second dimension channel lengths were maintained at $L_1 = 1\, \text{cm}$, $L_2 = 0.04\, \text{cm}$ and $L_3 = 5\, \text{cm}$, respectively. The resulting maximum current ratios between adjacent first dimension segments are shown in Fig. 5(a). Without backbiasing ($R_b/R_1 = \infty$), significant current asymmetries are revealed, and found to increase with the density of channels in the second dimension array. Even with the conservative selection of channel lengths used in this example, maximum current ratios are greater than half the asymptotic approximation given by eqn (3) for $R_1 \rightarrow \infty$. However, when the backbiasing channel resistance is increased to twice that of the second dimension channels ($R_b = 2R_2$), balanced injection is achieved and the current ratio is equal to unity, independent of the number of second dimension channels. In addition to eliminating current asymmetry in the first dimension channel segments, backbiasing can also serve to reduce current variations within the parallel second dimension channels themselves. As seen from Fig. 3, current variations within adjacent first dimension segments which feed a single second dimension channel approximately balance each other, such that a higher current in one segment is nearly offset by
a correspondingly lower current in the adjacent channel. Thus, despite the large current ratios between adjacent first dimension segments, current variations within the second dimension channels are relatively small. Regardless, these variations are entirely eliminated when 2x backbiasing is used. This is evident from Fig. 5(b), which shows the maximum ratio of currents across all second dimension channels as a function of the backbias channel resistance and number of second dimension channels. Without backbiasing, the current variation is less than 0.3% for a 3 channel design, and increases gradually as additional channels are added to the array. The introduction of backbiasing channels with $R_b = 2R_l$ eliminates the second dimension current variations, regardless of the number of second dimension channels in the array.

Sample transfer simulation and experimental validation

To evaluate the full sample injection process for a staggered 2-D chip design with and without backbiasing, numerical simulations were performed using FEMLab software (COMSOL AB, Stockholm, Sweden). In addition, multidimensional chips were fabricated, and sample transfer experiments were performed to provide validation of the simulation results.

For both simulations and experiments, the nominal system parameters are shown in Table 1. The sample mobility and buffer resistivity values given in this Table were measured experimentally to provide parity between simulations and experiments. The diffusion constant for SDS-complexed BSA was taken from the literature. Note that mobility of the SDS-complexed BSA sample used for experimental validation was found to be nearly identical within both free solution and LPA gel media, and so a single value was used for simulations. Note also that the analyte possesses a negative charge, requiring a negative bias to be applied to the injection channel reservoirs and reversing the current directions in Fig. 2.

Simulation results using a staggered chip design without backbiasing are shown in Fig. 6(a). Non-uniformities for the uncompensated chip can be seen clearly 3 s after applying the injection bias. For each pair of first dimension channel segments feeding a given second dimension channel, sample within the segment further from the chip center mobilizes into the second dimension more rapidly than the segments closer to the center, as expected from the electrical network model results (Fig. 3). The degree of asymmetry is so severe that even after 5 s (not shown), sample continues to gradually elute into the second dimension, resulting in long and poorly defined injection plugs. In addition, a small amount of sample continually leaks into the outermost second dimension channels due to diffusion and electromigration of excess sample from the ends of the first dimension channel. In contrast, simulation results using a chip design employing 2x backbiasing are shown in Fig. 6(b). In this case, uniform injection is observed within each second dimension channel, with well-defined and equal-length injection plugs, and no leakage into the outermost channels.

Experimental validation was performed using a 2-D chip fabricated with the same geometry used for simulations. Analyte consisting of pre-labeled BSA complexed with negatively-charged SDS was used to evaluate injection performance due to its relatively low diffusivity. Fluorescent images acquired during the sample transfer process are presented without backbiasing in Fig. 7(a), and with 2x backbiasing in Fig. 7(b). These experimental results, which are highly repeatable, match well to the simulation results for both cases, with 2x backbiasing providing highly uniform sample transfer between the dimensions.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Nominal system parameters for simulations and experimental validation</th>
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<tbody>
<tr>
<td>Parameter</td>
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<tr>
<td>Injection channel length ($L_1$)</td>
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<td>First dimension channel segment length ($L_s$)</td>
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<td>Second dimension channel length ($L_2$)</td>
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<tr>
<td>Sample diffusion constant$^{22}$</td>
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</table>
Fig. 7 Experimental sample transfer results using a staggered 2-D chip (a) without backbiasing, and (b) with 2x backbiasing. Backbiasing channels are not shown.

**Angled channel design for reduced sample tailing**

Although the elimination of injection non-uniformity using 2x backbiasing leads to a reduction in effective sample plug length within the second dimension channel array, the injected plugs still exhibit long tails which can impact the minimum achievable plug length. The tails visible in the last panels of Figs. 6(b) and 7(b) result from the geometry of the interdimensional intersections, which produce large gradients in the vertical component of the electric field within the first dimension channel. These gradients in turn generate variations in sample velocity during injection. Modification of the intersection geometry to minimize electric field gradients is one approach towards reduced sample tailing. While there are several ways to achieve this goal, here we consider a simple modification based on rotating each first dimension channel segment by an angle $\alpha$ from the original channel orientation (see inset in Fig. 8). The benefit of the angled channel design can also be seen from Fig. 8, which plots the normalized electric field profiles along the y-axis defined in the inset. Without the angled geometry ($\alpha = 0^\circ$), the electric field varies by a factor of 65, with the lowest value at the point furthest from the second dimension channel inlet ($y = 0$). This value drops to less than 5 when the channel angle is increased to $45^\circ$. As a result, a 2-D chip employing a $45^\circ$ angled channel design will exhibit significantly reduced tailing.

Experimental results validating this statement are shown in Fig. 9. This test was performed using a chip with 2x backbiasing channels to ensure uniform sample transfer during injection. The backbias channels were designed with the same cross-sectional channel dimensions and gel medium as the second dimension channels, but with twice the length. Compared with the straight channel case (Fig. 7), sample tailing is negligible for the angled channel design due to the more uniform electric field. The improved electric field uniformity also provides faster sample transfer and smaller plug lengths at the heads of the second dimension channels. When taking into account the tailing observed using the straight channel design, sample plug length was reduced an average of 34% using the angled channel design.

Fig. 8 Electric field strength profile within first dimension channel along center line of second dimension channel with varying channel angle ($\alpha$). Channel geometry is shown inset.

While tailing can be further minimized by increasing the angle of the first dimension channel segments, other factors including electric field uniformity and sample dispersion during the first dimension separation must also be considered. Increasing the angle may also impact the ease of initial sample injection and lead to unwanted extrusion of sample out of the first dimension channel during sample introduction. For cases where large channel angles may unduly affect the first dimension separation performance, smaller angles can still provide measurable benefits during sample transfer while minimizing the impact on the first dimension separation.

**Summary**

Microfluidic technology is unique in its ability to seamlessly integrate large numbers of fluidic elements into a single compact package. As the complexity of microfluidic separation systems
grows, it is important to consider design issues which set these platforms apart from traditional capillary or slab gel systems. The staggered two-dimensional chip design evaluated here is largely based on multidimensional slab gel platforms, but with very different design considerations resulting from the discretized nature of the microfluidic elements. In particular, the combination of 2x backbiasing and angled first dimension channel segments has been shown to substantially improve sample transfer performance in these systems.

Proper backbiasing can completely eliminate non-uniformities during both sample transfer and second dimension separations, and also prevent leakage of residual sample from the outer regions of the first dimension channel. Optimized backbiasing channels, with twice the resistance of a single second dimension channel, can be realized in a number of ways. In the present work, all channels used the same cross-sectional dimensions, and so the length of the backbiasing channels was doubled to realize 2x backbiasing. Alternately, channel width or depth could have been reduced by half while maintaining identical lengths between the backbiasing and second dimension channels. Backbiasing can also be performed by directly applying appropriate biases to each reservoir at the ends of the first dimension microchannel. However, this approach requires accurate knowledge of buffer conductivities within both dimensions and thus adds unnecessary uncertainty compared to on-chip backbiasing channels, which ensure uniform injection regardless of the buffer conditions or separation media used in each dimension.

Angled first dimension channel segments are also a valuable design element for realizing optimized sample transfer in staggered 2-D microfluidic chips. The angled channel design provides a simple approach for minimizing sample plug tailing and initial sample plug length within the second dimension channels, thereby offering improved peak capacities for the overall system. While this paper has focused on the sample transfer process using a single homogeneous analyte, we have also performed full 2-D separations using chips combining both 2x backbiasing and angled channel design elements, using microfluidic isoelectric focusing (μIEF) in the first dimension and gel electrophoresis in the second dimension. While this work is ongoing, it is worth noting that the angled channel design does not substantially affect focusing resolution during μIEF. Moreover, the uniform injection and minimized tailing provided by this design feature is providing improvements in the full 2-D separation performance even for relatively complex samples such as cell lysates.

Although electrokinetic injection was assumed in this work, the same conclusions apply to hydrodynamic injection. This can be seen by considering pressures and flow velocities as analogs of electrical voltages and currents, respectively, in the provided derivations. Thus, if pressure-driven separations such as liquid chromatography were employed within the second dimension, the use of 2x backbiasing and angled first dimension segments would still provide optimal sample transfer. In this sense, the proposed design modifications represent a universal approach for improving sample transfer performance, and thus separation efficiency, for any combination of separation mechanisms used within a staggered 2-D chip.

Acknowledgements

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