Effects of liquid conductivity differences on multi-component sample injection, pumping and stacking in microfluidic chips

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Received 25th April 2003, Accepted 20th June 2003
First published as an Advance Article on the web 7th July 2003

As an increasing number of processes are being integrated into Lab-on-a-chip devices, there is an increasing need for flexible and accurate sample manipulation techniques for effective transport and separation. Conductivity differences between running buffer and analyte samples can arise as a product of on-chip processing, or by design. The two situations studied here are sample pumping (where bulk transport is increased and separation of charged analytes is delayed using a relatively high conductivity sample), and sample stacking (where bulk transport is decreased and separation of charged analytes is expedited using a relatively low conductivity sample). A recently developed dynamic loading method for on-chip sample injection in a straight-cross channel configuration is applied here to both pumping and stacking cases. A key characteristic of the dynamic loading method is the ability to inject samples of high concentration density and uniformity of any length. By employing the conductivity differences alone, the effectiveness of either sample transport or sample separation are shown to improve over the uniform conductivity case. Then it is demonstrated that increasing the sample length, through dynamic loading, greatly increases the effectiveness of sample pumping, evidenced in an eight-fold increase in peak height as well as a decrease in total sample length at a downstream detector. Dynamic loading in the sample stacking case was shown to also increase peak intensity height (three-fold) in rapid separations. These results demonstrate that the dynamic loading technique, used in conjunction with strategic conductivity differences, significantly extends the capabilities of microfluidic chips.

1. Introduction

The integration of preparatory, reactive, and post-processing functions with analytical techniques is central to the lab-on-a-chip concept. The ability to integrate these processes on a microfluidic chip is one of the most important advantages of on-chip devices. Concentration gradients, and corresponding gradients in electrical conductivity, arise naturally in this integrated framework. Conductivity differences can be used to facilitate sample manipulation in discrete, multi-component samples.4–13

The use of electroosmotic flow in microfluidic chips is often preferred over pressure-driven flow because of the degree of control it allows, and the characteristic plug-like velocity profile it exhibits. Unlike pressure-driven flow, however, the net velocity of an individual ionic species in an electroosmotic flow is a combination of the bulk fluid velocity and the specific electrophoretic velocity of the species. An initially discrete multi-component sample is thus separated into bands based on the charge-to-mass ratios of the individual species contained. The rate of separation of the sample can be changed using a sample with an electrical conductivity differing from that of the running buffer. Although the sample and choice of buffer are typically determined by the application, the relative buffer concentrations can often be varied (to some extent) to enhance or inhibit separation. An understanding of these systems is also required in less ideal cases where extremely high or extremely low sample concentrations are necessitated by the application. Conductivity differences alter the shape of the electric field, which is the driving force behind both the electroosmotic bulk flow and the electrophoretic velocity of individual species. In most lab-on-a-chip applications the channel length greatly exceeds the sample length, and thus the sample velocity is dictated primarily by the electroosmotic flow rate in the running buffer (regardless of the electroosmotic velocity developed at the sample/wall interface). Thus the bulk fluid velocity developed by electroosmosis is constrained by the conservation of mass requirement. The electrophoretic velocity of a charged species in the sample, however, is not constrained, and responds directly to the local electrical field gradient. The two cases of interest are sample pumping and sample stacking.4 In the pumping case, the sample conductivity, \( \lambda_s \), is higher than that of the running buffer, \( \lambda_o \), and the electric field strength in the sample, \( E_s \), is lower than in the running buffer, \( E_o \). The result is reduced electrophoretic velocities in the sample region, and hence a reduced rate of separation. Although the electroosmotic velocity in the sample, \( v_{eo,s} \), is likewise reduced, pressure forces induced by the comparatively high running buffer electroosmotic velocity, \( v_{eo,o} \), pull the sample along. The opposite is observed in the stacking case (field amplified stacking). In the stacking case, separation in the sample is enhanced using a sample with a lower electrical conductivity than that of the running buffer. The increased electric field leads to higher electrophoretic velocities in the sample region. The mean velocity of the sample, however, is constrained by the comparatively slow electroosmotic velocity in the running buffer. This combination results in rapid separation of species in a relatively slow moving sample. A key aspect of the stacking case is that it is possible to obtain peaks with concentrations of charged components higher than in the original sample solution. Increasing the peak height allows for more accurate detection, and in some cases extends the analytical capabilities of microfluidic chips.

Miniaturization of analytical devices can offer many benefits, however, the detection of analytes is often more difficult in Microsystems due to the characteristically small sample volumes and low concentrations employed.4 Conventional off-line concentration methods do not lend themselves well to microscale quantities of material, and in general it is desirable to...
integrate preparation steps on-chip as much as possible. For this reason, on-line sample preconcentration through sample stacking is of particular interest, and there has been somewhat more interest in stacking than in pumping applications. To achieve significant sample concentration for either pumping or stacking, many studies have employed larger samples. In contrast to focused samples employed for direct separation by capillary zone electrophoresis, stacking on chips was first demonstrated by Jacobson and Ramsey using a gated injection scheme. Due to the circulations generated, they suggested that a compromise between stacking enhancement (due to either increased conductivity difference or sample size) and separation efficiency must be reached. Haab and Mathies employed offset twin-T chip configurations where the sample and buffer run adjacent in the dispensing channel for a short distance. Unfortunately, the running of buffer and sample together in this way tends to dilute the sample. To avoid this, Lichtenberg et al. developed a new injector (essentially an offset twin-cross). Although more complicated, the new configuration was shown to successfully resolve the adverse effects of sample pinching in the offset twin-T. Maintaining sample purity is of particular importance because the degree of stacking or pumping achievable is critically dependent on the difference in conductivity of the injected sample relative to that of the running buffer. Any dilution of the sample during injection results in a reduction in the conductivity difference and a decrease in performance. A new three-step technique for discrete sample injection in straight-cross microfluidic chips was presented recently. The technique is a variation on the pinched valve injection. With the addition of an intermediate dynamic loading step in which sample is pumped directly into the intersection and three connecting channels. The three-step process is shown in the image sequence in Fig. 1. A key feature of this technique, especially in the context of pumping and stacking, is the ability to inject well-defined samples of high concentration density. The dynamic loading method has been demonstrated using only conductivity-matched liquids. Although the method is well suited to pumping and stacking cases, these applications were not investigated. A second key feature of this loading technique is that samples of any axial length can be injected, and in contrast to offset twin methods the sample size is not coupled to the chip geometry. This capability enables the effects of sample size (a critical parameter in both pumping and stacking cases) to be studied systematically through varying the dynamic loading period.

In this paper, the on-chip injection and electrokinetic transport of samples differing in conductivity from the running buffer are investigated through fluorescence-based visualization. A sample containing both a neutral dye (rhodamine-B) and negatively charged dye (fluorescein) is employed. While the charged dye responds to the field gradients induced by the conductivity difference between the sample and running buffer, the neutral dye simply tracks the location of the original sample buffer. Both electroosmotic pumping and stacking cases are studied. The coupled flow phenomena inherent in these situations are investigated and discussed in the context of microfluidic chip applications.

2. Materials and methods

2.1 Imaging apparatus

The sample employed was a fluorescent dye (or mixture of fluorescent dyes), and a fluorescent epi-illumination video microscope was employed to visualize the process. The dye was excited by a continuous flood of blue light provided by a single-line, 200 mW, 488 nm argon laser (American Laser Corp.), through a 32×, NA = 0.3 microscope objective (Leitz). The received signal was split by the dichroic mirror (510 nm LP/short-reflecting) and passed through an additional filter (515 nm LP) and a camera mount with 0.63× magnification before reaching the camera. The chip was clamped to a precision 3-axis stage. The two horizontal positioning axes were used to position the field of view, and the vertical axis to focus the microscope. Such arrangements are flexible, but care must be taken to ensure that the objective remains sufficiently insulated from the electrodes.

2.2 Voltage control

The three-step process requires independent control of three potential levels for each of the four electrodes (12 settings). Here, a high voltage power supply (Glassman PS/EH20R02.0) was used in conjunction with a custom-made voltage controller. The timing of each switch was controlled via a digital delay generator (Stanford Research Systems). The voltage controller was programmed for default outputs corresponding to the steady-state loading step. Rising edge triggers from the delay generator commenced dynamic loading and dispensing steps. Switch frequencies up to 200 Hz were verified, indicating a transition delay of under 2.5 ms. Since the transition delay after each trigger is expected to be consistent, the critical dynamic loading period, t_{DL}, between triggers can be specified with considerably less uncertainty (estimated here < 1 ms).

2.3 Image acquisition and processing

Images were captured and saved on the computer at a rate of 15 Hz. A progressive scan CCD camera (Pulnix, TM-9701) was used to avoid image defects due to field–field interlacing. The acquired images had a resolution of 640 × 484 pixels and an 8-bit dynamic range. This pixel matrix corresponded to a viewed region of 350 × 416 μm. The camera orientation was carefully adjusted before each run such that the pixel grid was aligned with the coordinate directions of the intersection. Digital image processing was performed to remove any non-uniformities present in the imaging system and to relate the pixel intensity values to dye concentration. A background noise signal was subtracted, and bright field image normalization was performed for each image. These images were then smoothed with a distance-based kernel, and scaled by a single factor such that the image series filled the grayscale range.
2.4 Microfluidic chip

The glass chips used here were manufactured by Micralyne (Edmonton, Canada). The channels are 20 μm deep (at the centre) and 50 μm wide (across the top), and the reservoirs are 2 mm in diameter. The D-shaped channel cross-section is a product of the manufacturing technique. The sample was loaded into reservoir R1, the sample waste reservoir was R3, and the buffer focusing reservoirs were R2 and R4. The lengths of the channels from the intersection to the reservoir are 5 mm, 4 mm, 80 mm, and 4 mm for channels 1, 2, 3, and 4 respectively.

2.5 Chemicals and chip preparation

The fluorescent dyes employed here were rhodamine-B (478.68 MW) as supplied by Fisher, and fluorescein (332.31 MW) as supplied by Molecular Probes. The dyes were dissolved in sodium carbonate buffer of pH = 9, also used as the running buffer. To pull these solutions through each channel, a vacuum

was applied to R3 using a 30 ml syringe. A tapered 1000 μl pipette tip was cut to the appropriate diameter and friction fitted to join the syringe to the chip.

3. Results and discussion

The period over which the dynamic loading voltages are applied is termed the dynamic loading period, $t_{DL}$. For a given solution/chip combination and electrode potential settings, the dynamic loading period determines the axial length of the injected sample. The case of zero dynamic loading period, $t_{DL} = 0$, corresponds to the standard pinched injection described previously. The degree of stacking or pumping is dependant on both the initial size of the sample, and the conductivity of the running buffer relative to that of the sample buffer, $γ$. The dye concentrations, sample and running buffer ionic strengths, and normalized electrode potentials employed in each case are summarized in Table 1.

The effect of varying the conductivity difference (keeping initial sample size approximately constant) is shown in Fig. 2. Iso-intensity profiles obtained during the dispensing of a two-dye mixture are shown for each of the three cases: pumping, uniform conductivity, and stacking ($γ = 0.2, γ = 1, \text{and } γ = 5$ respectively). No dynamic loading was used, and the pinch-injected samples were of similar initial size. The two dyes in the relatively high conductivity sample ($γ = 0.2$, Fig. 2a) do not separate, and in that sense, the sample was effectively ‘pumped’. However, the sample is severely distorted by mismatch in electroosmotic velocities, and non-uniformity in the cross-stream direction due to the nature of the pinched injection. The effects of both these factors can be mitigated using larger initial sample lengths, as will be shown later. In contrast to the pumping case, the two dyes separate readily in the uniform conductivity case ($γ = 1$, Fig. 2b). The two dyes are even more rapidly separated in the relatively low conductivity sample ($γ = 5$, Fig. 2c). The fluorescein band in this case is thinner than that in the uniform conductivity case.

Table 1. Run parameters for each case studied. All potentials listed were normalized with the maximum applied voltage

<table>
<thead>
<tr>
<th>Figures</th>
<th>Uniform conductivity</th>
<th>Pumping case</th>
<th>Stacking case</th>
</tr>
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<tbody>
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<td>2b, 3, 4</td>
<td>2a, 5, 6</td>
<td>2c, 7, 8</td>
<td></td>
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<tr>
<td>Relative conductivity, $γ$</td>
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<td>0.2</td>
<td>5</td>
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<tr>
<td>Rhodamine-B concentration/μM</td>
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<td>100</td>
<td>200</td>
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<tr>
<td>Fluorescein concentration/μM</td>
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<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Running buffer ionic strength mM</td>
<td>25</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>Sample buffer ionic strength mM</td>
<td>25</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>Maximum voltage applied, $V_{max}/V$</td>
<td>1415</td>
<td>1415</td>
<td>1415</td>
</tr>
</tbody>
</table>

Normalized reservoir potentials:

| Loading step | | Dynamic loading step | | Dispensing step |
|--------------|-----------------------------|-----------------------------|-----------------------------|
| 1 | 1.000 | 0.964 | 0.014 |
| 2 | 1.000 | 0.911 | 0.124 |
| 3 | 0.000 | 0.000 | 0.000 |
| 4 | 1.000 | 0.911 | 0.000 |

Fig. 2. Iso-intensity contour plots during the dispensing of a two-analyte sample with: (a) a relatively high conductivity sample (pumping case, $γ = 0.2$); (b) a sample with conductivity matching the running buffer ($γ = 1$); and (c) a relatively low conductivity sample (stacking case, $γ = 5$). No dynamic loading was employed. Contours are plotted at 5 even intervals.

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due to field amplified sample stacking. Fig. 2 highlights the very significant role conductivity differences play in electrokinetic sample transport. Although the effectiveness of sample transport (pumping case) and sample separation (stacking case) are shown to improve somewhat over that of the uniform conductivity case, the injections shown in Fig. 2 are far from ideal. The underlying phenomena and the effect of initial sample length for each case (uniform conductivity, $\gamma = 1$, sample stacking, $\gamma = 5$, and sample pumping, $\gamma = 0.2$) will now be investigated in turn.

When the conductivity of the sample matches that of the running buffer ($\gamma = 1$) neither sample stacking or pumping occurs. In such cases, individual analytes in the sample move independent of the original sample-buffer solution at a velocity equal to the summation of the bulk electroosmotic velocity and their specific electrophoretic velocity. Upon dispensing, neutral rhodamine and negatively charged fluorescein are separated readily as shown in the sequence of iso-intensity contour plots in Fig. 3. Regarding the size of the analyte bands, the fluorescein band remains in the intersection much longer than the rhodamine band. This positioning results in a loss of fluorescein into the sample and sample waste channels due to the application of pull-back (or equivalently ‘cut-off’) voltages. In addition, it would be expected that the fluorescein band would be smaller than the rhodamine band due to the electrophoretic injection bias in the pinched valve scheme. When a dynamic loading step was applied, the sample stream was electroosmotically pumped directly into the dispensing channel. The effect of dynamically loading the same sample mixture as employed previously (Fig. 3), under the same conditions ($\gamma = 1$) is shown in Fig. 4. Images with samples at similar locations were chosen from the dispensing step of each run and processed identically. Dynamic loading is shown to increase the peak height of both analytes. As dynamic loading time was increased, however, there was little increase in the peak height of the rhodamine band as it approached a plateau concentration value equal to that of the original sample stream. The dynamic loading step, where neutral ions travel much faster in the dispensing channel than anions, amplifies the injection bias, previously reported for pinched injections. This bias was evidenced by the larger increase in rhodamine bandwidth (compared to that of the fluorescein) accompanying a dynamic loading period of just 0.1 s. In this uniform conductivity system, the benefit of increased peak height obtained with dynamic loading is at least partially offset by the cost of delayed separation due to increased band length.

To facilitate the electroosmotic pumping case for reduced separation, a fluorescein/rhodamine sample mixture was prepared with buffer five times more conductive than the running buffer, $\gamma = 0.2$ (run details in Table 1). The dynamic loading of the high conductivity sample is shown in Fig. 5. In order to pull the high conductivity sample to the intersection, the focusing potentials in the steady state loading step were decreased from that of the previous run. In the dynamic loading step, electroosmotic flows of the running buffer pulled the comparatively slow sample stream into the dispensing channel. Upon application of the dispensing voltages, the sample takes on a curved shape in the dispensing step, characteristic of the pumping case. No separation was observed in the limited field of view shown in Fig. 5c. This result is in contrast to the rapid separation observed in the $\gamma = 1$ case (Fig. 3).

To investigate the effect of initial sample length in the electroosmotic pumping case, the microscope was repositioned.

![Fig. 3](image-url)

**Fig. 3** Iso-intensity contour plots of the injection of a two-analyte sample with conductivity matched to that of the running buffer ($\gamma = 1$): (a) the steady-state loading step ($t = 0$ s), (b) the dispensing step ($t = 4/15$ s), and (c) later in the dispensing step ($t = 8/15$ s). No dynamic loading was employed. Contours are plotted at 10 even intervals.

![Fig. 4](image-url)

**Fig. 4** Centreline axial intensity profiles obtained during the dispensing of a two-analyte sample with conductivity matched to that of the running buffer ($\gamma = 1$). Profiles for dynamic loading times of $t_{DL} = 0$ s, 0.1 s, and 0.2 s are superimposed with corresponding iso-intensity contour plots inset.

![Fig. 5](image-url)

**Fig. 5** An image sequence of the three-step dynamic loading injection process of a two-analyte sample of higher conductivity than the running buffer (pumping case, $\gamma = 0.2$). The dynamic loading time was $t_{DL} = 0.3$ s.
loaded samples at the point of detection are shown inset. The first injection employed no dynamic loading \( (t_{DL} = 0) \), the following injections employed increased dynamic loading times and the final injection was a repeat of the no dynamic loading case. Note that although the scale of the time axis is accurate, detection was paused after each dispensing step to allow the analytes to return to the intersection and fill into the sample waste reservoir. In the first and last case, which employed the shortest samples, the sample had begun to separate into two bands, the rhodamine peak at the detector before the fluorescein peak. With a dynamic loading step of \( t_{DL} = 0.1 \) s, the peak height more than doubled, although the sample is shown to be fronting in time (or ‘trailing’ in space). This fronting is primarily due to cross-stream diffusion in the wake of the convex sample shape, not to be confused with fronting due to mobility differences between the analyte and that of the running buffer co-ion.\(^{21}\) Due to diffusion, the characteristic shape is only barely noticeable in the inset images of the samples as they crossed the detection point in Fig. 6. As the initial sample length was increased (by increasing \( t_{DL} \)), the peak height increased greatly. The band length, however, was not similarly increased. Although the \( t_{DL} = 0.8 \) s sample was the largest sample injected, it was the sample with the smallest band length at the detector, and thus the most effectively ‘pumped’. This is because in multi-component, high conductivity samples, the separation rate is coupled to the concentration density of the sample buffer. Detailed study of the coupled equations governing velocity, concentration, and electrical potential fields in these systems requires numerical simulation,\(^{12}\) and only an overview is given here. Briefly, dilution occurs at the sample ends due to an internal circulation generated by the mismatch in electroosmotic mobilities between the sample and the running buffer. The rate at which that dilution affects the average concentration of the sample is a function of sample length. Thus, larger samples experience a reduced rate of overall change than shorter samples under similar circumstances. By maintaining the conductivity difference, larger samples maintain lower internal electrical potential gradients and hence experience less broadening due to the separation of analytes. The similarity of the first and last \( (t_{DL} = 0) \) runs verifies that the increase in pumping effectiveness observed, evidenced in an 8-fold increase in peak height and a decrease sample length, was attributable to the dynamic loading of the high conductivity sample.

To facilitate the electroosmotic stacking case for enhanced sample separation, a fluorescein/rhodamine sample mixture was prepared with buffer one-fifth as conductive as the running buffer, \( \gamma = 5 \) (run details in Table 1). In Fig. 7, iso-intensity contour plots and centerline intensity profiles are plotted at 4/15 s intervals during the dynamic loading and separation process. In the steady state loading step (Fig. 7a), the sample is transported to the intersection by the relatively high electro-osmotic velocity in the sample channel, and experiences a relatively high electrical field strength. With application of the dynamic loading potentials, the sample pumps into the dispensing channel for \( t_{DL} = 0.2 \) s (Fig. 7b). The sample entering the dispensing channel is noticeably depleted in fluorescein. This depletion is due to the high electrical field strength and reduced bulk flow in the sample stream. Essentially, the sample-stacking mechanism is being applied to the whole sample channel, and fluorescein is being ‘stacked’ toward the sample reservoir. Also apparent is an M-shaped centerline intensity profile in Fig. 7b (right). It is suspected that during the steady-state loading step, the fluorescein concentration was increased near the intersection as was found by Jacobson.\(^{13}\) This local highly-concentrated portion was split during the dynamic loading step forming the M-shaped profile. With application of the dispensing potentials (Fig. 7c), the fluorescein in the sample rapidly stacked to the rear (left) of the sample, creating a large peak in intensity. Because of the symmetry of dynamic loading, the stacked peak is formed to the left of the intersection \((x < 0)\), partially insulated from the sample and sample waste channels. In contrast, the neutral rhodamine did not stack, marking the location of the low conductivity sample buffer. In addition, the front of the rhodamine band exhibited the concave shape characteristic of the stacking case. As the fluorescein and the rhodamine further separate, some fluorescein was consumed by the sample and sample waste channels due to the pull-back voltages applied. Although the analytes were totally separated in Fig. 7c, the two separate bands were distinguishable almost immediately after the application of the dispensing potentials (Fig. 7e). This rapid separation is due to the high field strength developed in the sample.

To investigate the role of dynamic loading in sample stacking, injections with varying dynamic loading times were performed. Images from each run (taken at a similar time period

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Fig. 6  The signal recorded (at a location 550 μm downstream of the intersection) over time through a series of seven injections. The sample contains two-analysts and has a higher conductivity than the running buffer (pumping case, \( \gamma = 0.2 \)). The first injection employed no dynamic loading \( (t_{DL} = 0) \), the following injections employed increased dynamic loading times and the final injection was a repeat of the no dynamic loading case. Images of the dynamically loaded samples at the point of detection are shown inset.
following the application of the dispensing potentials) were processed identically. The superimposed centerline intensity profiles are shown in Fig. 8. Being neutral, rhodamine behaved here as in the uniform conductivity case, reaching a plateau concentration value equal to that of the original sample stream. The peak height and band length of the fluorescein band, however, are shown to increase significantly with dynamic loading. A dynamic loading period of $t_{DL} = 0.3$ s resulted in a three-fold increase in fluorescein peak height over that produced through sample stacking without dynamic loading. Also, as the dynamic loading time is increased the sample length increases, moving the resulting location of charged analyte stacks further from the intersection. Imposing this distance has the benefit of insulating the analyte from the sample and sample waste channels, further increasing peak height.

For the purposes of this study, altering the dynamic loading period was a logical and convenient way to investigate the role of the sample size in each case. It is important to note that this flexibility also extends to applications of this procedure, which could involve a tremendous variety of buffers, analytes, and conductivity differences. In most situations the optimal set of injection parameters, analyte concentrations and conductivities is not known nor is it often economical to obtain it by calculation. The ability to alter initial sample volume from one injection to the next allows for rapid on-line optimization, or ‘tuning’, of the injection for a given application.

4. Conclusions

Conductivity differences between sample and running buffer streams can greatly influence the transport of individual analytes in electrokinetically driven microfluidic systems. The two situations studied here were sample pumping (where bulk transport is increased and separation of charged analytes is delayed using a relatively high conductivity sample), and sample stacking (where bulk transport is decreased and separation of charged analytes is expedited using a relatively low conductivity sample). It was shown that by employing the conductivity differences alone, the effectiveness of either sample transport or sample separation was improved over the uniform conductivity case. Then it was demonstrated that increasing the sample length, through dynamic loading, further increased the effectiveness of sample pumping, evidenced in an eight-fold increase in peak height as well as a decrease in total sample length at a downstream detector. Dynamic loading in the sample stacking case was shown to increase peak height (three-fold) in rapid separations. The success of these applications of

![Fig. 7](image)

**Fig. 7** Plot sequence of the dynamic loading and dispensing of a two-analyte sample of lower conductivity than the running buffer (stacking case, $\gamma = 5$). Iso-intensity contour plots at 4/15 s intervals are shown on the left, and corresponding centerline axial intensity profiles are shown on the right. The dynamic loading time was $t_{DL} = 0.2$ s. Contours are plotted at 5 even intervals.
dynamic loading was attributed to the ability to inject concentration dense samples of any length. Although these processes would benefit from a formal optimization, it was demonstrated that the dynamic loading technique used in conjunction with strategic conductivity differences significantly extends the capabilities of microfluidic chips.

Acknowledgements

The authors gratefully acknowledge the support of a Research Grant of the Natural Sciences and Engineering Research Council (NSERC) of Canada to D. Li, an NSERC scholarship to D. Sinton, and an Ontario Graduate Scholarship to L. Ren. Financial support from Glynn Williams, through a postgraduate scholarship to D. Sinton is also gratefully acknowledged.

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Fig. 8 Centreline axial intensity profiles obtained during the dispensing of a two-analyte sample of lower conductivity than the running buffer (stacking case, g = 5). Profiles for dynamic loading times of tDL = 0 s, 0.1 s, 0.2 s, and 0.3 s are superimposed.