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Sample stacking in laboratory-on-a-chip devices

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Abstract

Sample stacking is a very important sample concentration technique. It has been used widely in capillary electrophoresis (CE). There are many different stacking techniques. One of the most popular techniques is called ''field-amplified sample stacking'' where an electric field discontinuity is set up across a concentration boundary. Charged analytes will then automatically stacked due to velocity changes after they cross the concentration boundary. There are several different strategies to perform sample stacking in microfluidic laboratory-on-a-chip devices. One could simply inject a plug of low concentration buffer containing sample into a channel surrounded by high concentration buffer. The electric field is then applied to stack the sample and move the whole plug into the separation channel. One could also stack the sample in a side channel adjacent to the separation channel. The disadvantage of this sample stacking technique is the difficulty in control of the precise location of stacked sample. We present a new sample stacking technique applied specifically to microfluidic laboratory-on-a-chip devices. Up to hundreds of fold increases in sample concentration can be achieved. We have also combined this stacking with electrophoretic separation in the same device.

Keywords: Sample stacking; Chip technology

1. Introduction

Microfluidic devices and systems have been developed that provide substantial advantages in terms of analytical throughput, reduced reagent consumption, precision of data, automatability, and integration of analytical operations and miniaturization of analytical equipment [1–5]. These devices and systems gain substantial benefits from operating within microscale range where analytes are carried out on sub-microliter, and even sub-nanoliter quantities of fluid reagents. Because these systems operate on such small scales, they use substantially smaller amounts of precious reagents, are able to mix and react materials in much shorter time frames, can be performed in small integrated systems, and are far more easily automated.

While microfluidic devices and systems have a large number of advantages, the one area where they suffer from a distinct disadvantage over conventional scale analysis is the sample is usually present at very low concentration in very small volumes. Often, this amount of analyte may fall near or below the detection threshold for the analytical system. In conventional scale operations, material can be provided in much larger volumes and concentrated prior to analysis, using conventional off-line concentration methods. These conventional concentration methods, however, do not lend themselves to microscale quantities of material. Accordingly, it would be desirable to be able to perform an on-line sample concentration operation on microfluidic devices to

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increase the detection sensitivity for an analyte of interest.

There are many different sample concentration techniques used in capillary electrophoresis (CE). Three most widely used techniques are field-amplified sample stacking (FASS) [6–8], isotachophoresis (ITP) [9,10], and solid-phase extraction (SPE) [11,12]. ITP involves use of at least two distinctive electrolyte buffers, a leading electrolyte and a terminating electrolyte, to confine the samples in between. Although ITP could be a very powerful sample concentration technique, unfortunately it never gains the popularity due to its complexity. SPE is a physical concentration technique that requires a specific binding of the analyte to an immobilized phase. However, it is not trivial to implement SPE on a microfluidic device.

FASS on the other hand is one of the simplest sample concentration techniques in CE. There are many different modes of operation in FASS [7]. Generally speaking, it involves injecting a plug of samples in low concentration buffer into a background buffer having the same composition but higher concentration. In addition to different concentration, other mechanisms such as pH and viscosity changes have been proposed to provide velocity difference between the two regions. In any event, an electric field discontinuity is generated across a concentration boundary. Charged analytes will then automatically stacked due to velocity changes after they cross the concentration boundary. This ''stacking'' effect results in a substantial concentration of the analyte at the concentration boundary. By combining this effect with the facility of controlled fluid movement through integrated channel networks in microfluidic devices, one can effectively concentrate, then further manipulate a particular charged material.

Different strategies to perform sample stacking in microfluidic laboratory-on-a-chip devices have been proposed by several groups [13–16]. One could simply inject a plug of low concentration buffer containing sample into a channel surrounded by high concentration buffer. The electric field is then applied to stack the sample and move the whole plug into the separation channel. One could also manipulate the plug or move it out to the side channels. The disadvantage of this sample stacking technique is the difficulty in control of the precise location of stacked sample. In addition, the extent of stacking is limited by the original injected sample. We will present a new sample stacking technique applied specifically to microfluidic laboratory-on-a-chip devices. Up to 100 folds of increases in sample concentration could be achieved. We have also combined this stacking with electrophoretic separation in the same device.

2. Chip designs

As we alluded earlier, sample stacking in a laboratory-on-a-chip device could be achieve by injecting a plug of sample in low concentration buffer into the channels filled with high concentration buffer. The stacked sample could then be switched into another channel for further processing or separation. A variety of different channel layouts can be used for sample stacking. The complexity and design of different channel networks is often dictated by the desired manipulations to the sample prior and subsequent to the sample concentration step. Fig. 1 shows a schematic diagram of sample stacking process in a simple cross in a laboratory-on-a-chip device with inherent electroosmotic flow. In Fig. 1a, a plug of sample in low concentration buffer is initially loaded into device filled with high concentration buffer by some hydrodynamic means. High voltage is then applied across the loading channel to perform sample stacking as shown in Fig. 1b. The stacked analytes then migrated into the injection cross by the combination of electroosmotic and electrophoretic flow. Finally, high voltage is applied to the orthogonal separation channels as shown in Fig. 1c to inject the sample for further separation and processing.

In a standard uncoated glass chip, there is usually an appreciable amount of elecotroosmotic flow which make it extremely difficult to control the exact location of the stacked sample. In addition, to prevent generating back-pressure due to mismatch of electroosmotic flows in different regions and to achieve an optimum stacking, one has to limit the length of injected low concentration sample plug. This severely restricts the amount of stacking and concentration one can achieve.

If the chip is coated to eliminated electroosmotic flow, the concentration boundary will be stationary except for dispersion caused by diffusion. To per-

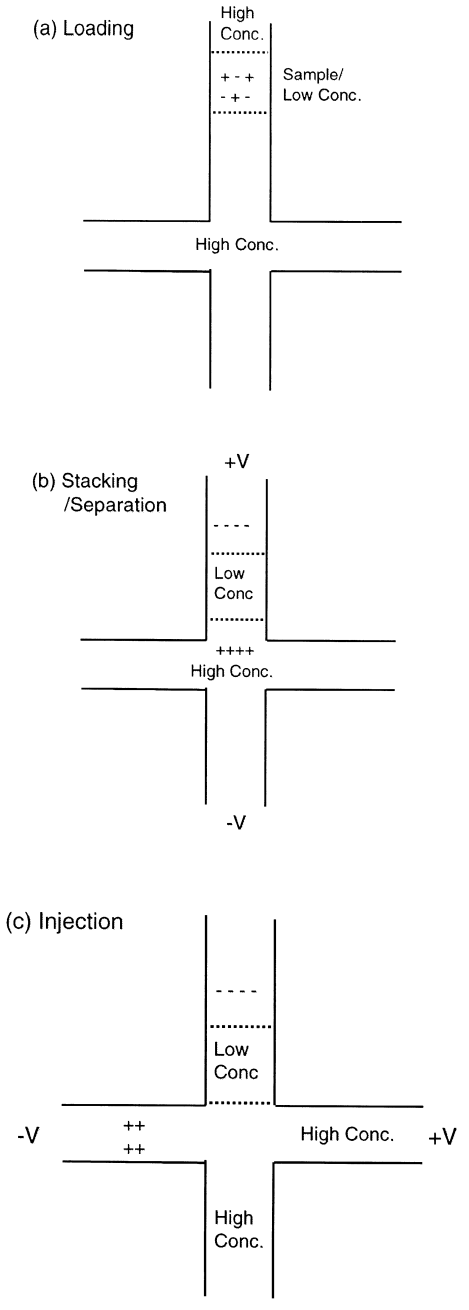

Fig. 1. A schematic diagram showing sample stacking in a typical laboratory-on-a-chip device with electroosmotic flow. (a) Analytes in low concentration buffer is injected into the channels filled with high concentration buffer. (b) High voltage is then applied across the loading channel and the stacked sample migrated into the injection cross. (c) High voltage is finally switched to the cross channels for further processing or separation.

form sample stacking in such a device, one would like to have the loading channel filled with low concentration buffer and the remaining three channels filled with high concentration buffer as shown in Fig. 2a. Ideally, The concentration boundary should be located very close to the injection cross to make the stacking and injection processes fast and efficient.

To set up this stationary boundary, one needs to be able to control the flow from all channels appropriately. The channels in the chip are filled with the low concentration buffer first. The high concentration buffer is then transported into all but one of these channels from two side arms. This could be done by providing a vacuum from the bottom channel while applying another vacuum source on the top well to hold the flow from the top channel. We achieve this by using a laboratory-made multiport pressure/voltage controller and an advanced flow control algorithm. The result is a stationary boundary as shown in Fig. 2a. The sample could then be continuously injected and stacked after the concentration boundary. Theoretically, there is no limit on the amount of stacking. It is simply determined by the ratio of field strength, or concentration in a first-order approximation, between two regions.

It is very difficult to make the concentration boundary stationary in the cross channels shown in Fig. 2. A very small electroosmotic flow will shift the concentration boundary and ruin the whole process. A better chip design for static sample stacking is shown in Fig. 3. In this design, in addition to a cross-section, one more side channel is used to provide the concentration boundary. Not only is an efficient sample stacking could be achieved, this design also tolerates a small amount of electroosmotic flow. Fig. 4 shows a photolithography mask specially designed for the stacking purpose. This chip contains a five-port cross injection/separation device. The marks along the long separation channel measure the distances from the injection intersection.

3. Experimental

3.1. Elimination of hydrodynamic and electroosmotic flow

Elimination of electroosmotic flow can be accom-

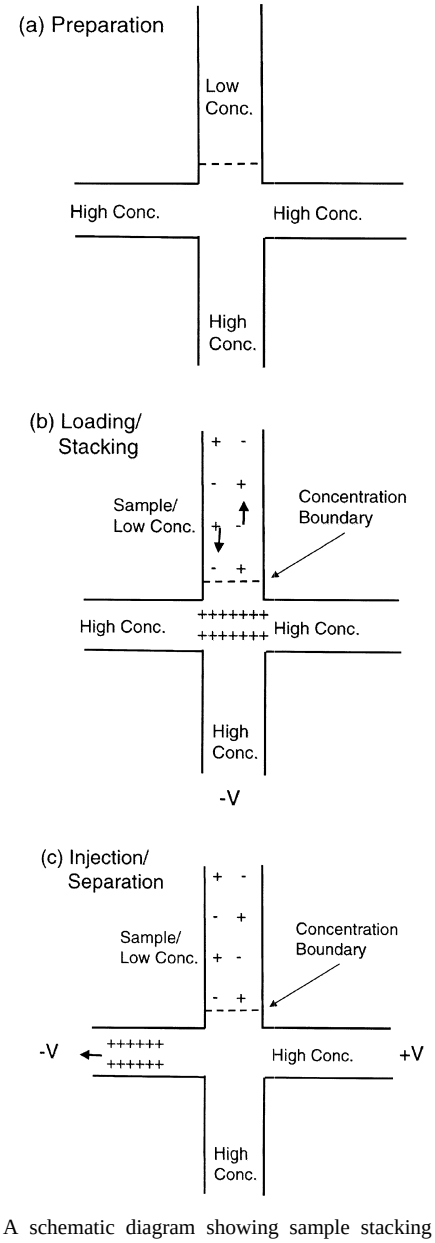

Fig. 2. A schematic diagram showing sample stacking without electroosmotic flow in the same device as shown in Fig. 1. (a) The device is prepared such that the loading channel is filled with low concentration buffer and the remaining three channels are filled with high concentration buffer. The concentration boundary is located very close to the injection cross. (b) High voltage is then applied across the loading channel and the charged analytes are continuously injected and stacked after the concentration boundary into the injection cross. (c) High voltage is then switched to the cross channels to inject the concentrated analytes into the separation channel.

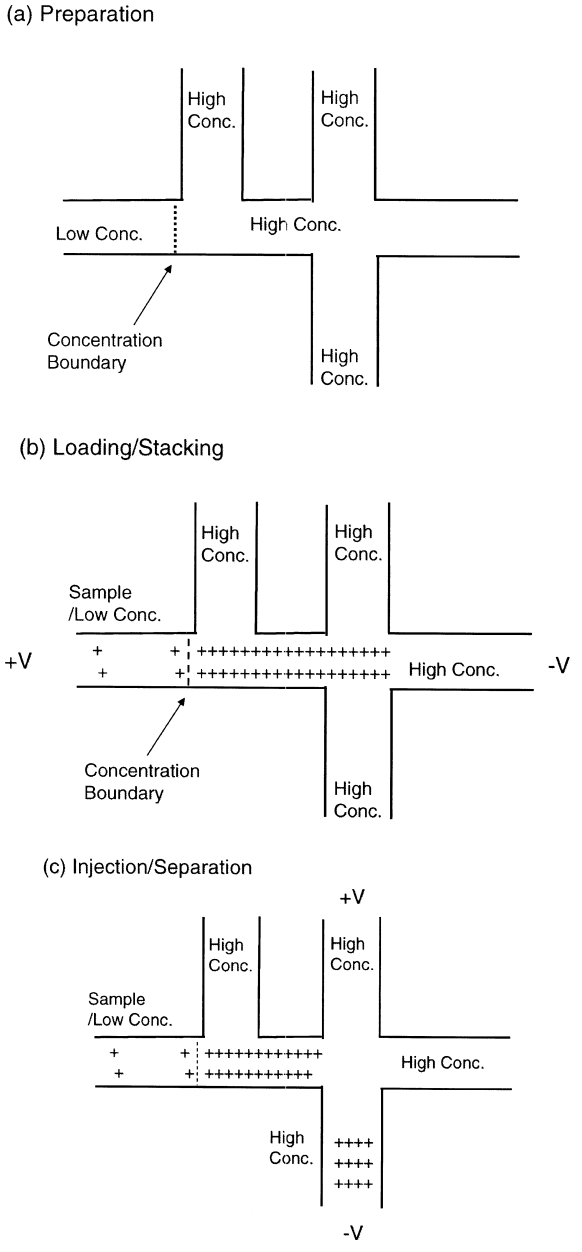

Fig. 3. A schematic diagram of a better chip design for static sample stacking.

plished by several means as well. Typically, one can mask the charge on the wall by coating the channel's interior surface and consequently to eliminate electroosmotic flow. One could also adjust the pH or viscosity of the buffer to reduce flow. In our

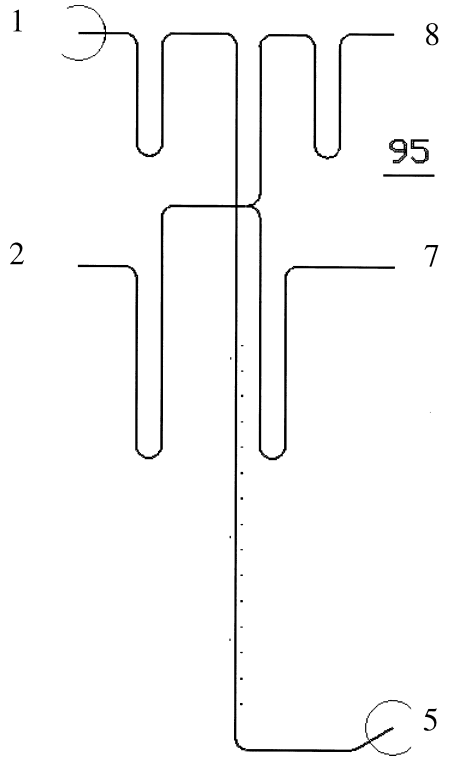

Fig. 4. Mask design of the stacking chip.

experiment, the chips were coated by the poly(allyl-glycidyl ether-co-*N*,*N*-dimethylacrylamide) (pDMA/E) copolymer purchased from Polysciences (Warrington, PA, USA). The procedure of coating to eliminate the electroosmotic flow is as follows:

1. Rinse and fill the chips with filtered 1 *M* NaOH for 20 min.
2. Flush the chips with filtered deionized (DI) water.
3. Fills the chips with filtered 0.1% pDMA/E polymer solution and let the chips sit for 1 h.
4. Flush the chips with filtered DI water.
5. Dry the chips by vacuum.

3.2. Reagents

The uninterested termini of the channel segments were connected to fluid reservoir in the surface of the devices. Several different ratio of conductivity were used in this experiment.

High conductivity buffer: 200 m*M* NaCl in 100 m*M* 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.5.

Low conductivity buffer: 1 m*M* NaCl in 0.5 m*M* HEPES, pH 7.5.

Extra low conductivity buffer: 0.2 m*M* NaCl in 0.1 m*M* HEPES.

Two dyes, a fluorescein sodium salt and a fluorescein labeled protein (FL–Leu–Gly–Arg–Ile–Val) from Molecular Probe (Eugene, OR, USA), with concentration about 5 μ*M*, are then mixed into the low conductivity buffer, to serve as a detectable charged sample materials

3.3. Apparatus

A modified Nikon microscope system with a Multiport pressure/voltage chip manifold was used for the stacking experiment

In most microchip devices, electrokinetic forces, either electroosmotic or electrophoretic flow is used to move samples around. Electrokinetic means are convenient and match very well with microchip devices. While electrokinetic material transport systems provide numerous benefits in the microscale movement, mixing and aliquoting of fluids, it will be a great advantage if one could add hydrodynamic flow control to the system.

The flow-rate in the laboratory-on-a-chip devices is usually in the order of nanoliters per second. To accurately control such a tiny amount of flow with incompressible liquid is extremely difficult. We have designed a multiport pressure/electrode controller capable to apply different pressure settings to multiple reagent wells on a chip. The detailed description of this system will be published elsewhere. Briefly speaking, the system consists of eight syringe pumps that could be addressed and operated individually. The output of each syringe is then connected to a Tee which is connected to a pressure sensor that monitor the pressure reading of the syringe pump. The other end of the Tee is connected to a polyether ether ketone (PEEK) or PTFE tubing connected to a pressure manifold to interface with the laboratory-on-a-chip devices. Since the flow impedance of the control line could be made several orders smaller than the impedance of the channels, the pressure change generated by the syringe pump will transfer completely to the microfluidic device. In addition,

very fast time constant could be achieved due to the small impedance of the control line.

Although it is straightforward to control the pressure above the wells, it is much more complicated to measure and monitor the flow-rate, especially in the micro-scale level. To solve the problem, we have devised a computer algorithm to translate the hydrodynamic flow-rates into controllable pressures for each well. The flow control program is written in LabView (National Instruments, Austin, TX, USA) and the high voltage is control by a separate program and hardware designed by Caliper Technologies (Mountain View, CA, USA).

3.4. Conductivity ratio measurement

1. Fill the chip with the low conductivity buffer.
2. Apply 2000 V between well 1 and well 5, and record the resulting current (current L)
3. Replace with the high conductivity buffer. Apply 250 V and repeat the same measurement (current H).

The conductivity ratio is then calculated from the current ratio and normalized with the applied voltage.

3.5. Experiments with no stacking

1. Add 35 μl of the low conductivity buffer in wells 1, 2, 5, and 7.
2. Add 35 μl of the sample in well 8.
3. Load the sample into the intersection by flowing it from well 8 to well 2 electrokinetically.
4. Electrokinetically inject to well 5 by switching flow from well 1.
5. Detect fluorescence signal at the second marker.
6. Repeat load/injection by looping the script steps.

3.6. Experiments with stacking

1. Add 35 μl of the high conductivity buffer in wells 1, 2, 5, and 7.
2. Add 35 μl of the sample in well 8.
3. Generate the high/low conductivity boundary by the eight-port pressure controller; and release the pressure after the boundary formed.
4. Stack and load the sample into the intersection by flowing it from well 8 to well 2 electrokinetically.
5. Electrokinetically inject to well 5 by switching flow from well 1.
6. Detect fluorescence signal at the first marker.
7. Repeat load/injection.

4. Results

The pictures in Fig. 5 demonstrate proof-of-concept of this static stacking idea. The experiment was performed in a chip with four channel segments connected to a simple injection cross. The termini of the channel segments were connected to fluid reservoir in the surface of the devices. Two buffers were used. The high conductivity buffer was 100 m*M* Hepes with 200 m*M* NaCl and the low conductivity buffer was 0.5 m*M* Hepes with 1 m*M* NaCl. Due to impurities and other contamination, the measured conductivity ratio between high and low buffer is about 140:1 instead of expected value 200:1.

The entire channel network was initially filled with the low conductivity buffer by placing the buffer into one reservoir and allowing it to wick throughout the channel network. High conductivity buffer was then placed in the remaining reservoirs. The chip was then placed into a multiport pressure controller interface, which simultaneously controls the pressure applied at each of the four reservoirs. By knowing the channel geometry and viscosity of the buffer, one can calculated the required pressures to achieve the desired flow-rate. The system flowed the high conductivity buffer through two of the channel segments into the intersection and out through a third channel segment while applying a slight flow in from the fourth channel to maintain the low conductivity buffer interface. This resulted in high conductivity buffer in three of the four channels and low conductivity buffer in the fourth channel, with the interface between the two buffers immediately adjacent to the intersection. Similar approach could be used to prepare the static interface in complicated networks with more than four channel segments.

In our first experiment, we added the fluorescein dye in the low concentration buffer. The concentration boundary is barely visible as shown in Fig. 5a. However, this stationary boundary was also confirmed by adding a high concentration of neutral rhodamine dye.

After preparing the static interface in the four-channel segment network, an electric field was

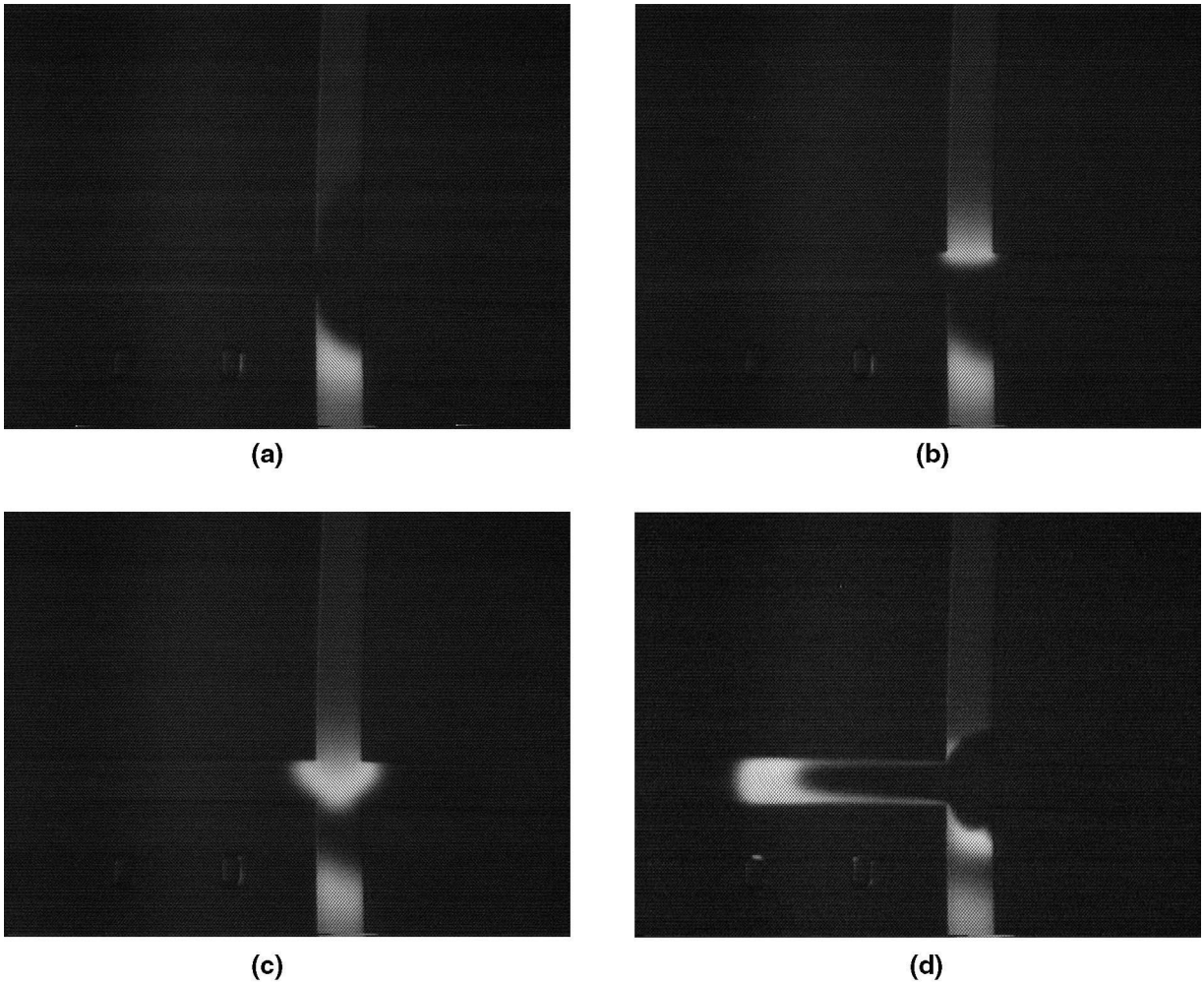

Fig. 5. (a) Background signal before the sample dye reaches the concentration boundary in the region near the injection cross. (b) Sample just cross the concentration boundary into high concentration. (c) Stacked sample migrates into the injection cross. (d) Stacked sample migrates into the injection cross.

applied through the low conductivity buffer and at least one of the high conductivity channels. The field cause a substantial concentration of the charged fluorescein dye at the interface between the low and high conductivity buffer regions as shown in Fig. 5b. In a typical experiment, we obtain an increase of concentration about a factor of 100 calculated from the dye signal. This agrees closely with theoretically predication of 140 given the conductivity ratio between the two fluids.

To prevent any remaining electroosmotic flow in the opposite direction, we applied a slight pressure above the sample reservoir to make sure the concentration boundary would remain in the injection cross. The stacked sample is then migrated and diffused into the injection cross as shown in Fig. 5c. In this figure, the sample stacks in a circular shape due to the positive hydrodynamic flow mentioned above. The sample sometimes also diffuses into the horizontal separation channels due to current leakage. We could compensate this leakage and focus the sample by setting small current flow toward the staking channel from the separation channels.

One could then switch the electric field from the

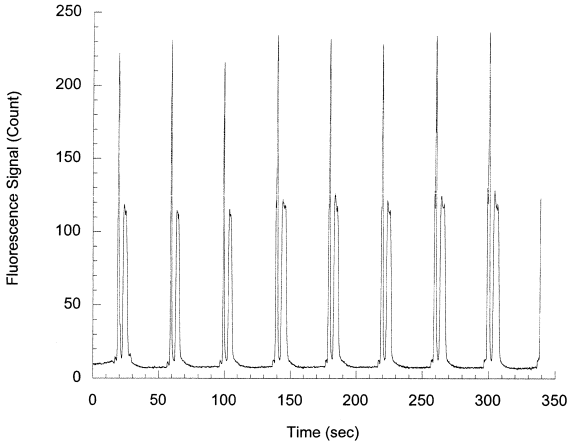

Fig. 6. Electropherogram on the separation of two dyes without stacking.

loading/stacking channels to injection/separation channels. A short and concentrated plug of sample is then injected into the third channel segment to perform separation as shown in Fig. 5d.

Similar concentration and separation processes could be performed on more complicated channel networks. To demonstrate this application, two dyes, a fluorescein salt and a fluorescein-labeled protein, were used as the detectable charged sample materials in a chip design as shown in Fig. 4. Fig. 6 is an electropherogram for the separation of two dyes without stacking and Fig. 7 is one with stacking. The signal increases by roughly a factor of 100 with

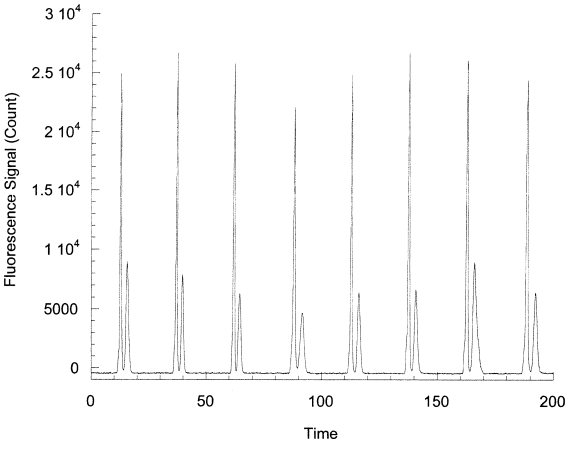

Fig. 7. Electropherogram on the separation of two dyes after stacking.

stacking. Although there are other issues needed to be investigated further, such as peak shape and reproducibility, one could clearly see the advantages of using sample stacking in a microfluidic device.

5. Conclusion

We have shown the possibility of performing static sample stacking in laboratory-on-a-chip devices by coating the device to eliminate electroosmotic flow. Sample could then be stacked very close to the injection cross. Depending on the ratio of conductivity between low and high concentration buffer, a factor of several hundreds of sensitivity enhancements could easily be achieved.

This stacking process is very efficient and fast for a sample in a low conductance buffer. However, it is well known that field-amplified sample stacking is very sensitive to the salt concentration in the sample. Most real samples contain high salts, which can limit the amount of stacking one can achieve. Although we have shown the proof-of-concept of this static-stacking process, it will be interesting to apply this technique to real samples and perform further studies. We are also interested in using other techniques such as ITP or isoelectric focusing to perform sample concentration on laboratory-on-a-chip devices. By taking advantages of the unique features of microfluidic systems, such as coupled columns and advanced injectors, one could take many innovative approaches to improve the detection limits of real life samples and make the miniaturization a reality.

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