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Short communication

Sample flow switching techniques on microfluidic chips

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Abstract

This paper presents an experimental investigation into electrokinetically focused flow injection for bio-analytical applications. A novel microfluidic device for microfluidic sample handling is presented. The microfluidic chip is fabricated on glass substrates using conventional photolithographic and chemical etching processes and is bonded using a high-temperature fusion method. The proposed valve-less device is capable not only of directing a single sample flow to a specified output port, but also of driving multiple samples to separate outlet channels or even to a single outlet to facilitate sample mixing. The experimental results confirm that the sample flow can be electrokinetically pre-focused into a narrow stream and guided to the desired outlet port by means of a simple control voltage model. The microchip presented within this paper has considerable potential for use in a variety of applications, including high-throughput chemical analysis, cell fusion, fraction collection, sample mixing, and many other applications within the micro-total-analysis systems field.

Keywords: Microfluidics; Electrokinetic forces; Flow switching

1. Introduction

Lab-on-a-chip devices involve the miniaturization and integration of various chemical processes onto a single chip. The literature contains many reports of the miniaturization of traditional laboratory systems onto single microchip substrates (Manz et al., 1990; Gravesen et al., 1993). Micro-total-analysis systems (μ-TAS) combine the operations of sample preparation, mixing, separation and detection. Recently, significant progress has been made in developing microfluidic component, such as mixers (Moorthy et al., 2001; Oddy et al., 2001) and flow detectors (Schrum et al., 1999). Various rudimentary microfluidic systems have also been demonstrated (Norlin et al., 1998; Fu et al., 2004). Compared to their large-scale counterparts, these systems reduce the cost and increase the throughput of bio-analytical assays. Continuous sample injection is an essential requirement for bio-analytical assays. Accordingly, the aim of the present study is to develop a microfluidic device capable of providing a high-throughput continuous injection of single and multiple samples.

When performing bio-analytical assays it is necessary to guide the sample fluid flow precisely to the specified outlet port. The purpose of such operations is to deliver accurate amounts of liquid to the reservoir where the biochemical reaction is to take place. In the past, researches were devoted to flow switch development in microfluidic systems. Various designs have been reported for microchip-based flow-switching devices in which some form of active structure was integrated with fluid-carrying microchannels. For example, Döring et al. (1992) demonstrated that a laminar flow could be steered into one of two outlet ports by using a thermal bimorph cantilever with a Si/Al bimetal structure. In an alternative approach, Lemoff and Lee (2000) developed an ac magneto-hydrodynamic (MHD) microfluidic switch, in which the Lorentz force was used to pump an electrolytic solution. In their design, the sample flow was switched between two outlets by integrating two ac MHD pumps with a Y-shaped fluidic circuit.

In the two studies cited above, an active structure was integrated with a system of microchannels to guide the sam-

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ple flow into the required outlet port. However, samples can also be guided into the desired outlet port hydrodynamically. For example, Blankenstein and Larsen (1998) reported that establishing a differential hydrodynamic pressure between the inlet ports provided the means to guide the sample flow to the appropriate outlet port. It was shown experimentally that the sample flow could be successfully directed into any one of five different outlets. Similarly, Lee et al. (2001a,b) also reported that single or multiple sample injections could be guided to the desired outlet port by means of hydrodynamic forces. Their design introduced the novel concept of sample flow pre-focusing to provide a precise fluid flow direction control.

However, the designs presented in the studies above required some form of micropump to drive the fluids. This requirement introduces both control and cost issues. In an attempt to overcome this problem, Fu et al. (2003) investigated experimentally the use of electrokinetic forces to control the fluid flow for bio-analytical applications in $1 \times N$ (i.e. 1 sample inlet port and N outlet ports) and $M \times N$ (i.e. M sample inlet ports and N outlet ports) microfluidic chips. In the proposed approach, electrokinetic forces were employed both to drive the sample flow and to achieve sample focusing and switching.

Although the designed presented in Fu et al. (2003) was capable of directing both single and multiple samples to the desired outlet port, its operation required an excessive number of voltage control points. Hence, the intention of the present study is to design a microfluidic chip capable of performing continuous single or multiple sample injection to specified output ports by applying appropriate electrical potentials to a minimum number of voltage control points. Details including the design, fabrication, detection and the condition of operation for microfluidic chips will be discussed in the following sections.

2. Experimental procedure

2.1. Design and fabrication

Fig. 1 shows a schematic representation of the current micromachined pre-focusing $M \times N$ flow switch incorporating two sample inlet ports (B and D) and five outlet ports (1, 2, 3, 4 and 5). The remaining inlet ports (A, C and E) in the inlet section are sample flow focusing channels, which are designed to control the width of the sample stream and to direct the sample to the appropriate outlet port. The flow-switching device integrates the fundamental phenomena of electrokinetic injection and valve-less flow switching on a single microfluidic chip. The basic operating principle of the proposed $M \times N$ device when switching a single sample is described as follows: (1) the sample is introduced into the inlet channel via the application of an electrical potential to the sample inlet channel, (2) the same electrical potential is applied to each of the focusing channels in order to generate

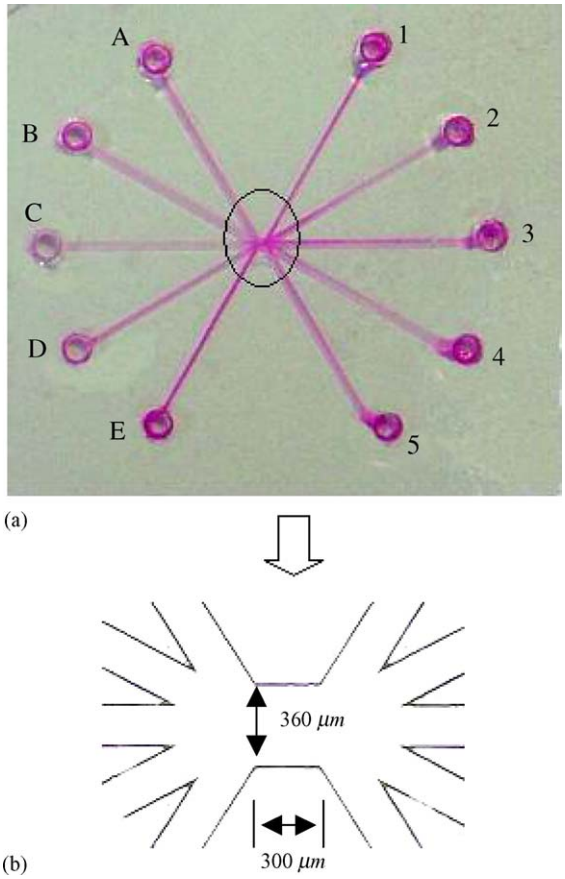

Fig. 1. (a) Each channel has size of width: 200 μm, depth: 20 μm and length: 1 cm. (b) Magnified view near the chamber between inlet and outlet channels.

sheath flows to focus the sample into a narrow stream, and (3) the outlet channels are electrically isolated or grounded, as appropriate, in order to drive the focused sample flow electrokinetically to the required outlet port. Note that the operating principle of the device when switching multiple samples is broadly similar in its approach.

This current microfluidic chip was fabricated on commercially available polished soda lime glass substrates with dimensions of Ø100 mm/1 mm using conventional photolithographic and chemical etching processes. The details of the fabrication process are presented by the current study group in (Lin et al., 2001), and hence the fabrication process is described only briefly here. Initially, the glass substrates were cleaned by boiling in a Piranha solution (H_2SO_4 (%)/H_2O_2 (%) = 3:1) for 10 min. The substrates were then rinsed in DI water and blown dry with nitrogen gas. In order to ensure the complete removal of residual water molecules, the substrates were baked on a hot plate at 100 °C for 3 min. The glass plates were coated with an AZ 4620 positive photoresist (PR) and a soft baking process was then conducted, resulting in a PR layer thickness of approximately 7 μm. The photomasks were generated using layout software (AutoCAD 2000) and were printed by a high-resolution laser printer. UV lithography was performed using a mask aligner (Suss MicroTec MA150CC.) with an exposure dose of G-line (436 nm) for

30 s. The PR development process was conducted by immersing the exposed substrates into a developer solution (AZ300k) for 135 s. After rinsing the exposed substrates in DI water and blowing them dry with nitrogen gas, they were hard baked at 150 °C for 10 min. They were then immersed in a BOE (buffered oxide etch) solution. During the etching process, the glass substrates were dipped in DI water every 5 min. After the etching process, the substrates were cleaned in a boiling Piranha solution for 10 min together with blank glass plates which had previously been drilled through with via holes. All of the plates were then rinsed in DI water. Finally, each substrate was bonded with a drilled glass plate via a fusion bonding process. Fig. 1a shows the geometry of the current microfluidic chip after completion of the photolithography process. The inlet and outlet channels have dimensions of width: 200 μm, depth: 20 μm and length: 1 cm. A magnified view near the chamber used for flow switching is provided in Fig. 1b.

2.2. Detection system

In the detection system arrangement, the fluid sample manipulations within the microchip are observed by mercury lamp induced fluorescence using a charge-coupled device camera (CCD, model SSC-DC50A, Sony, Japan). The experimental images are captured by an optical microscope (model Eclipse 50I, Nikon, Japan), filtered spectrally, and then measured by the CCD device. Temporal profiles of the switching process are obtained using a detection system similar to that presented in Alarie et al. (2000, 2001). In the present study, the sample was Rhodamine B (10^{-4} M) and the buffer liquid was 10 mM sodium borate (pH 9.2, Aldrich).

3. Results and discussion

3.1. Single sample flow switching

This study developed a simple voltage control model to establish a pre-focusing continuous sample injection system using the microfluidic chip described in the previous section. Table 1 (Case a–c) shows the electrical potential specifications required for the injection of a single sample into the five outlet ports of the microfluidic chip. Taking Case (a) for illustration purposes, the injected sample is to be directed into outlet port 1. Therefore, an electrical potential of 0.3 kV is applied to each of the five inlet ports (A, B, C, D and E), while outlet ports 2–5 are isolated and outlet port 1 is grounded. From electrokinetic theory, the driving force caused by the interaction between the net charge density and the applied electric field occurs only between the inlet ports and outlet channel 1. No driving force exists near outlets 2, 3, 4 and 5 since they are isolated and hence there is no electrical field gradient. Fig. 2a–c shows the continuous sample injection stream distribution for the three different output modes. In Fig. 2a, the sample is injected through channel B and is then directed electrokinetically to output channel 1. The sample is focused into a narrow stream by neighboring sheath flows supplied from input channels A and C. Note that the sheath flows not only focus the sample flow, but also prevent sample leakage. In this case, outlet channels 2–5 are isolated and outlet channel 1 is grounded. Hence, the focused sample flow is directed towards outlet channel 1. For illustration purpose of the present techniques applied to handle biological particles, polystyrene beads (10–12 μm) are used to mimic cells. These beads are placed in the flow and can be guided to appropriate outlet port by the same manipulation technique as well (see Fig. 2d). The other continuous single sample injection modes (Fig. 2b–c) operate in a similar manner. In practice, the fluid and particle can be continuously switched between the outlet ports by controlling the potential conditions, i.e. isolated or grounded, of the outlet channels. The experimental results show that the proposed microfluidic chip is capable of providing the continuous sample injection capability required to achieve a high throughput in bio-analytical assays systems. Only one power supply is required for such arrangements, therefore the number of voltage control points is minimum.

Table 1
Specification of electrical potentials for sample injection system

	Electric potential (kV)									
	A	B	C	D	E	1	2	3	4	5
Single-sample injection system										
(a)	0.3	0.3	0.3	0.3	0.3	0	×	×	×	×
(b)	0.3	0.3	0.3	0.3	0.3	×	0	×	×	×
(c)	0.3	0.3	0.3	0.3	0.3	×	×	0	×	×
Multi-sample injection system										
(e)	0.4	0.3	0.4	0.3	0.4	0	0	×	×	×
(f)	0.4	0.3	0.4	0.3	0.4	0	×	0	×	×
(g)	0.45	0.3	0.45	0.3	0.45	0	×	×	0	×
Mixing injection system										
(h)	0.35	0.3	0.35	0.3	0.35	0	×	×	×	×
(i)	0.35	0.3	0.35	0.3	0.35	×	0	×	×	×
(j)	0.35	0.3	0.35	0.3	0.35	×	×	0	×	×

(0) Ground; (×) isolation.

3.2. Multiple sample flow switching

To support the requirements of practical applications, this study extended the control voltage scheme described above for the $1 \times N$ continuous sample injection system to the case of multiple samples. A micromachined pre-focusing 2×5 continuous sample injection system was fabricated using the techniques described in Section 2.1. Fig. 3 presents a series of experimental views of multiple sample injection into specific outlet ports using the pre-focusing 2×5 sample injection system. As shown in Table 1 (Case e–g), the corresponding electrical potential control model is similar to that developed for the $1 \times N$ continuous sample injection system. In a similar approach to that described above for single sample flow switching, two outlet channels are grounded and the others are isolated such that electrokinetic driving forces are gen-

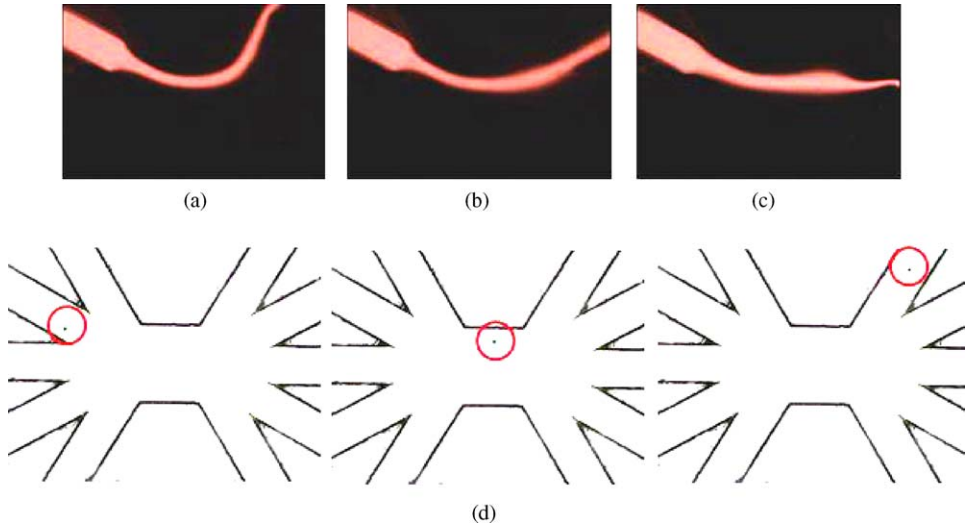

Fig. 2. The sample is focused into a narrow stream by neighboring sheath flows supplied from input channels A and C. By controlling the potential conditions appropriately, i.e. either isolated or grounded of the outlet channels, the fluid and particle can be guided into the desired outlet channel.

erated between the two inlet channels and the two desired outlets ports. In Fig. 3e, the sample is injected into inlet channels B and D and then electrokinetically focused into narrow streams by neighboring sheath flows in order to avoid sample leakage. In Fig. 3(g), the physical separation between the desired outlet ports is large, and hence the voltages applied to focusing channels A, C and E are slightly higher. The experimental results confirm that the proposed microfluidic chip can successfully direct two sample streams to the desired outlet ports by means of suitable manipulations of the applied control voltage.

In addition to performing continuous sample injection, the proposed pre-focusing $M \times N$ continuous sample injection system can also be applied to perform sample mixing. Table 1 (Case h–j) shows the electrical potential specifications required for the injection of two samples into one of outlet ports of the microfluidic chip. Taking Case (h) for illustration purposes, the injected sample is to be directed into outlet port 1. Therefore, an electrical potential of 0.35 kV is applied to inlet ports (A, C and E) and 0.3 kV is applied to inlet ports (B and D), while outlet ports 2–5 are isolated and outlet port 1 is grounded. Fig. 4 presents a series of experi-

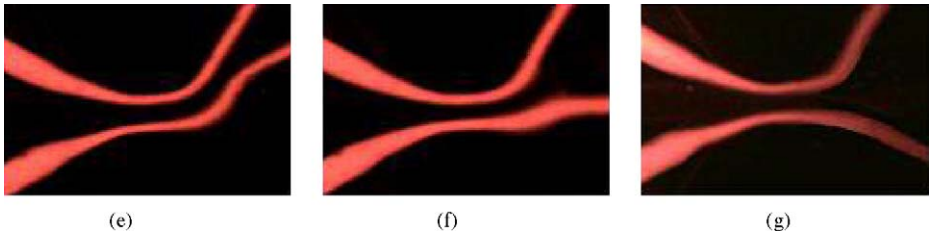

Fig. 3. Experimental results for different flow injection modes in multiple sample continuous injection system. Two outlet channels are grounded and the others are isolated such that electrokinetic driving forces are generated between the two inlet channels and the two desired outlets ports.

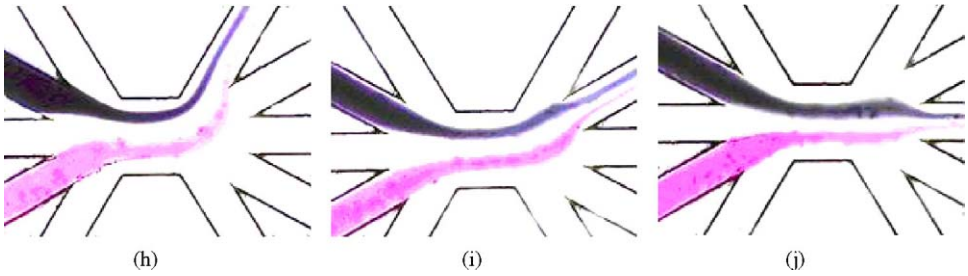

Fig. 4. The experimental results show two different sample streams being directed to a single outlet channel. The two streams are merged in the specified outlet channel and therefore fluid mixing takes place along streamwise direction.

mental results showing two different sample streams, one is fluorescent dye and another is black dye, being directed to a single outlet channel. The two different samples merges in the outlet channel and their subsequent mixing takes place along streamwise direction.

4. Conclusion

This paper has presented an experimental investigation into electrokinetic focusing flow injection for manipulating flow switching without using valve. The experimental results have shown that single samples and multiple samples can be focused into narrow streams by neighboring sheath flows, which not only constrain the fluid flow, but also prevent sample leakage. The pre-focused sample flow can be guided to the desired outlet port by establishing a ground connection at the desired port while isolating the remaining outlet channels. The experimental results have confirmed that sample flows and particles can be accurately controlled within the $1 \times N$ and $M \times N$ microfluidic devices by means of simple control voltage models using a minimum number of voltage control points. Additionally, the results have shown that two different sample streams can be successfully directed into a single outlet channel by the application of appropriate electrokinetic forces. Therefore, the developed $M \times N$ microfluidic system can support sample mixing applications. In conclusion, the microfluidic chips fabricated in this study are suitable for high-throughput chemical analysis, sample mixing, fraction collection, and many other applications in the field of micro-total-analysis systems.

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