Micro fluidic Biochips

Lung-Ming Fu, Department of Materials Engineering

Micro fluidic chips and lab-on-a-chip devices have attracted considerable research attention recently due to their increasing application in biomedical diagnosis and analysis procedures, including capillary electrophoresis, flow cytometry, polymerase chain reaction (PCR), DNA amplification, and protein analysis. Today's micromachining technology enables a network of micro channels to be fabricated on a single substrate of glass, PMMA (polymethylmethacrylate), PDMS (polydimethylsiloxane), PC (polycarbonate) or quartz. The resulting micro fluidic chips are capable of supporting a variety of different procedures, including sample injection, mixing, cell sorting and counting, chemical reactions and sample separation. Compared to conventional macro scopic devices, micro fluidic chips have the advantages of reducing human operation errors and sample amounts, shortened operation time, high performance and system stability. Such devices offer the potential for a parallel operation and / or integration with other miniaturized devices to accomplish the lab-on-chip and micro-total-analysis system (-TAS). Particularly, to avoid pollution in medical applications, it is imperative to develop novel, cheap and disposable micro fluidic biochips.

Recently, our researches in micro fluidic chip included injection and separation system, micro flow cytometer and development and application of micro fluidic reactors.

1. Injection and Separation Systems on a Micro fluidic Biochip

The injection and separation systems on a micro fluidic biochip are one of the key elements in the sample handling process. The chip characteristics determine the overall separation quality. Therefore, the injection system on a micro fluidic biochip design becomes an important issue. Many discussions relating to cross-injection systems on micro fluidic biochips can be found in the published literature. Although in the first injection run, such systems are capable of providing an ideally sized and orientated sample plug to the separation channel, sample leakage (Fig. 1) tends to occur from the upper and lower micro channels after several runs, and this phenomenon reduces the device detection performance. The study presents an experimental and numerical investigation into using low leakage injection techniques (double-L injection method, Fig. 2) to deliver sample plugs within micro fluidic biochips. Low leakage injectors designed with injection channels orientated at various included angles are designed and tested. Achieving high-resolution detection results in a chip-based micro fluidic device requires that the sample bands injected into the separation channel be of the correct shape and orientation. Therefore, this study also develops an integrated micro fluidic device which combines a low-leakage injection system with an expansion chamber at the inlet of the separation channel to deliver high-quality sample bands into the separation channel. This study provides a detailed description of the proposed high-resolution separation technique and adopts an experimental and numerical approach to evaluate the performance of the micro fluidic device with different expansion chamber configurations. It will be shown that the high-quality sample bands delivered into the separation channel through the expansion chamber with the optimal configuration significantly improve the detection resolution of the micro fluidic device, hence rendering it ideal for applications requiring a highly sensitive sample separation (Fig. 3)

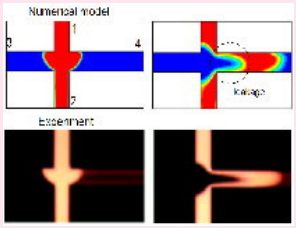

Fig. 1 Cross injection method

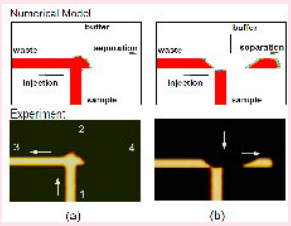

Fig.2 Double-L injection method

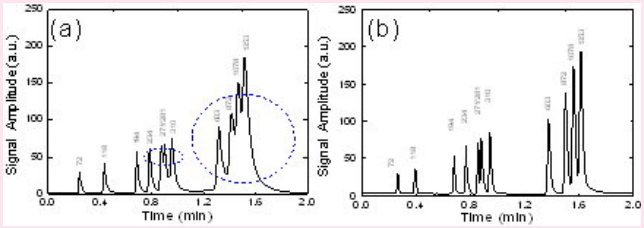

Fig. 3 Electropherogram of separation results for x-174 DNA sample using (a) traditional cross-form, and (b) double-L injection system with expansion chamber.

2. Micro Fluidic Flow Cytometer

Flow cytometry is a general method for analyzing micro-particles such as cells, bacteria and even euglena with high efficiency. It has been a popular diagnostic equipment for clinical and environmental applications. However, a well-trained technician and complicated optical equipment are required for traditional flow cytometry methods using laser induced fluorescence to detect the fluorescence-labeled cells/particles while performing the test. Therefore, this system may not meet the requirements for fast detection and for building a portable system such that the applications for clinical detection are hindered. For micro-flow cytometer, 2-dimensional hydrodynamic or electro kinetic focusing in the cell/particle counting in X-Y planar, they may be located at different depths and due to the lack of vertical focusing (Z-direction) in the micro channel. It will influence the detection performance of such flow cytometers. This study uses micro-electro-mechanical system (MEMS) technique to fabricate 3-dimensional focusing micro-flow cytometer (Fig. 4) in which cells/particles are concentrated in the center of the sample stream using a 2-D hydrodynamic focusing technique and a micro-weir structure (Fig. 5). The micro-flow cytometers also integrated an optical waveguides detection system and a conductivity measurement system for on-line cell/particle counting and sorting. The system can be used for fast counting of cells/particles, and then sorted using electric or hydrodynamic forces downstream.

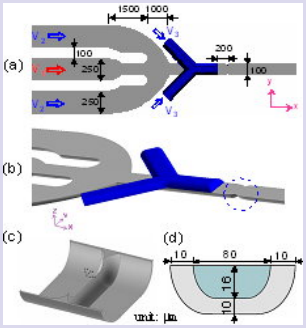

Fig. 4 (a) and (b) Schematic illustrations of micro-flow cytometer geometry seen from different directions, (c) 3D schematic of micro-weir structure, and (d) cross-section of micro-weir geometry.

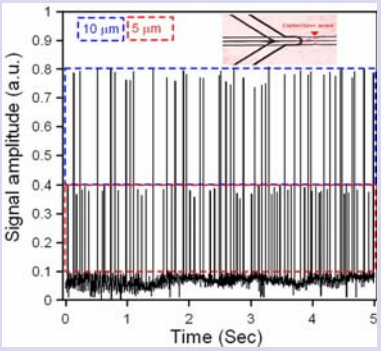

Fig. 5 Optical signals for sample stream containing mixture of 5 μm beads and 10 μm beads detected in micro-weir region of micro channel.

3 Micro Fluidic Reactors

Micro fluidic reactors have been widely applied in the fields of chemical, medical and biological analysis. They almost apply to require mixing of reagents with a sample for chemical or biological assay in advance. Our research presented a novel technique in which low-frequency periodic electro kinetic driving forces are utilized to mix electrolytic fluid samples rapidly and efficiently in a T-form, double-T-form, double-cross (Fig. 6), electro kinetic instability micro fluidic mixer and develops a novel passive micro mixer which utilizes self-rotation of the sample fluids from multiple injection channels to produce 3-dimensional vortexes in the circular mixing chamber at low Reynolds numbers (Fig. 7 and 8). Without using any additional equipment to induce flow perturbations, only a single high voltage power source is required for simultaneously driving and mixing the sample fluids, resulting in a simple, low-cost system for mixing purposes. The effectiveness of the mixer as a function of the applied electric field and the periodic switching frequency is characterized by the intensity distribution calculated downstream from the mixing zone. The present numerical and experimental results confirm that the proposed T-form, double-T-form double-cross, electro kinetic instability and 3-dimensional vortexes micro mixer has excellent mixing capabilities.

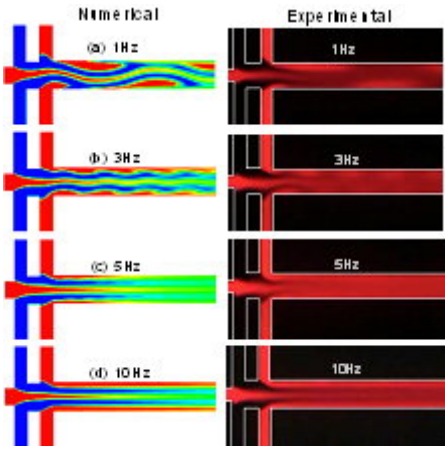

Fig. 6 Numerical and experimental results obtained for concentration distributions for switching frequencies in double-cross micro fluidic mixer

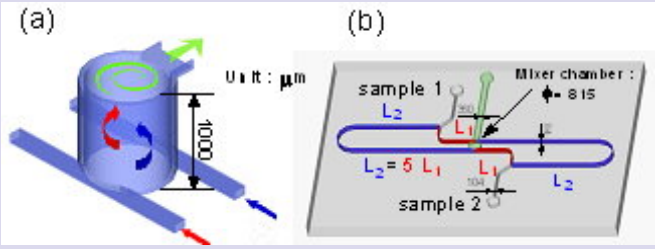

Fig. 7 Schematic illustration of circular micro chamber mixer geometry

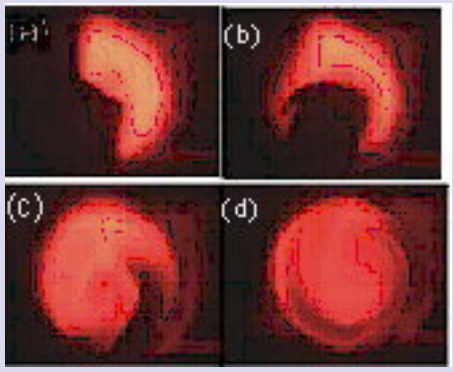

Fig. 8 Experimental flow images of flow rotation in circular micro-chamber mixer

3.1 A Rapid DNA Digestion System

This study presents a novel micro fluidic DNA digestion system (Fig. 9) which contains a pre-column concentrating region, a micro mixer for DNA-enzyme mixing, an adjustable temperature control system and a post-column concentration channel. Through the appropriate control of fixed and periodic switching DC electric fields, electro kinetic forces are established to mix the DNA and restriction enzyme samples and drive them through the device reaction column. The successful digestion of -DNA using Eco RI restriction enzyme is demonstrated. The DNA-enzyme reaction is completed within 15 min in the proposed micro fluidic system, compared to 60 min in a conventional large-scale system (Fig. 10). The results have shown that the speed of the -DNA digestion process is significantly increased in the developed micro fluidic system since the novel double-cross-form micro fluidic mixer ensures excellent mixing of the two reactants.

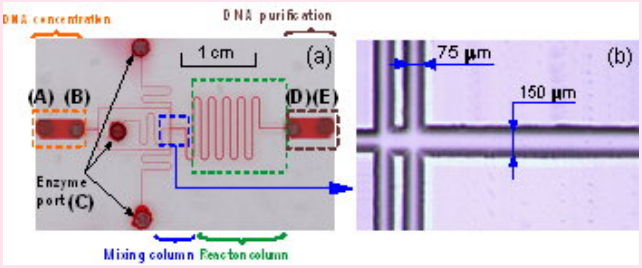

Fig. 9 Photograph of micro fluidic DNA digestion system.

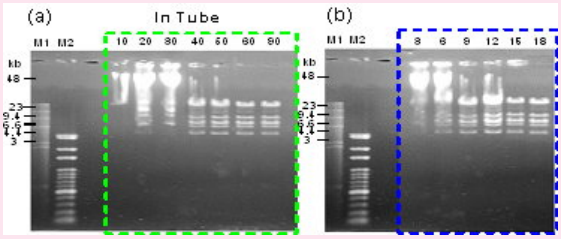

Fig. 10 (a) Slab-gel picture of electrophoresis results for DNA restriction experiment in a conventional tube system and (b) the slab-gel pictures of electrophoresis results for DNA restriction experiment using the microchip device.

3.2 Rapid Analysis of Formaldehyde in Food Utilizing Microfluidic Chip

We also uses micro fluidic chip with laser-induced fluorescence detection technique to detect inappropriate additives to food. In the experimental study, 4-amino-3-penten-2-one (Fluoral-P) and formaldehyde chemically interacted in a 3-D circular micro fluidic mixer (Fig.11) while the absorption and fluorescence intensity (of formaldehyde) were confirmed via rapid detection of fluorescence derivatization. When performing fluorescence intensity detection of formaldehyde derivatization, the fluorescence signal became flatter and the presence of noise was more likely as the formaldehyde concentration decreased. The limitation on this concentration was about 0.4 ppm (R2 = 0.9954)(Fig. 12).

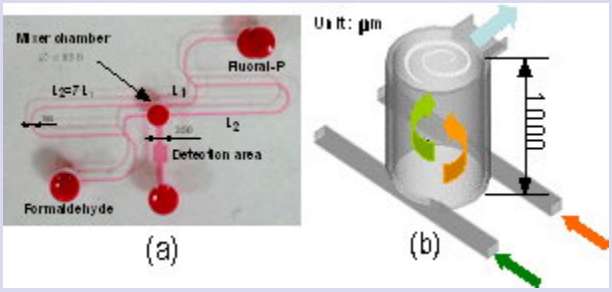

Fig. 11 Schematic illustration of micro fluidic chip for detection formaldehyde in food

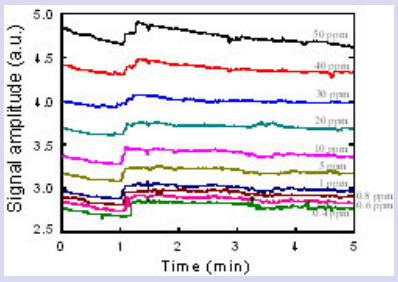

Fig. 12 The fluorescence signal expression with different concentration formaldehyde and Fluoral-P reaction using micro fluidic chip

3.3 Rapid Detection of Methanol in an Integration Microfluidic Chip

A rapid and simple technique was developed for detecting methanol with very small amount of sample by using PMMA (Polymethyl-Methacrylate) micro fluidic chip, which fabricated by a commercially available CO2 laser scriber. The micro fluidic chip designs are created using commercial layout software and are converted into the command signals required to drive the laser scriber in such a way as to reproduce the desired micro channel configuration on the surface of a PMMA substrate. The fluids are driven into the circular micro mixing chamber by means of hydrodynamic pumps from two fluid inlet ports. Each one-inlet port divides into 2 individual and different length channels tangent to a 3-Dimensinal (3-D) circular chamber to induce unbalance driving force for mixing purpose. The experimental results indicate that linearity expression R2 can approximate 0.936 using the proposed integrating micro fluidic chip when the 2 unit methanol oxidase (MOX) and basic fuchsin (BF, Schiff method) to detect methanol. Hence, the current device provides a valuable tool for rapid methanol detection, while its micro mixer system delivers a simple yet effective solution for mixing problems in the micro-total-analysis -systems field. ◆