

INTERNALLY TRIGGERD MULTISTEP FLOW SEQUENCERS USING CLEPSYDRA

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ABSTRACT

This paper presents an internally triggered multistep flow sequencer using centrifugal microfluidics. The results of experiments show successful demonstration of multistep flow sequencing by spinning the disk at constant spinning speed of 1500 rpm. We succeeded in to demonstrate two types of flow sequencing. One is timer sequencing using clepsydra which carryout four step flow sequencing autonomously. In the timer sequencing, timing of the all four steps are triggered by clepsydra. Other flow sequencing is chain flow sequencing which carryout three or five step flow sequencing autonomously. In the chain flow sequencing, a specific flow step is triggered by the end of flow step, which implemented just before the flow step.

KEYWORDS

autonomous, multistep operation, clepsydra

INTRODUCTION

Demand for the development of automation technology for the micro total analysis system (Micro TAS) is more and more increasing. The most standard approach to realize automated Micro TAS is to develop automated pneumatic control and syringe pumping systems using programmable controller. The fluidic chip is connected to the external systems and supplied with pressure and reagents. Therefore the total system requires many components, such as syringe pump, pneumatic pump, and many tubing. Therefore currently the automated Micro TAS intending to requires large external supporting systems, that making difficult to take Micro TAS out from laboratory use; the issue of chip-in-a-lab.

Centrifugal microfluidics is one of the promising ways to realize the automated Micro TAS, especially for the operation of numbers of systems. [1-6] Lai et al. developed a compact-disk-like device for enzyme-linked immunosorbent assay (ELISA) that can do automatic sequencing with a series of liquid injections for automatic multiple assays by just accelerating the spinning speed of the disk. [3] Fully automated immunoassay from whole blood is realized by using centrifugal microfluidics with wax valving. [4, 5] The actuation of valve to open the valve and also close the valve is implemented by irradiating the wax with infrared laser. A unique assay system using siphon valving has been developed. In the latest report, the flow sequencing and automated immunoassay was realized by using multiple siphon valves. [6] These studies shows relatively simple and automated immunoassay system by utilizing centrifugal force for the pumping, however all of them requires sophisticated controlling technique and chip fabrication. Therefore it expected that the development of the microchips and total system is still costly.

Besides, the development of autonomous controlling of Micro TAS has been another popular approach to develop simple, automated, compact, and low cost systems. Capillary driven pumping realize the simple pumping by utilizing surface wetting of the hydrophilic capillary. [7, 8] Therefore it does not require liquid pumping equipment. Power-free pumping is proposed. [9] In this method the evacuated PDMS is used for the pumping of the liquid. The gas inside channel gradually dissolves into the PDMS bulk texture and dissolved gas volume of liquid is sucked into the microchannel. These examples realize relatively simple and compact system however it requires some supporting hand operation to carryout complicated multi step operation. Recently, autonomous programmable control system for microfluidics is proposed. [10] In this study the fluidic system is designed based on the analogy of fluidic circuit with electronic circuit and multi step pneumatic valve and pumping control is controlled by simple pneumatic digital signal, and autonomous pump oscillation mode is switched. This is unique and having great potential to realize simple total system capable of complex multistep operation, however still have technical difficulties to develop compact system since bulky vacuum pump is required.

We have proposed a new autonomous flow sequencing method based on centrifugal microfluidics. [11] In this work two-step flow sequencing is demonstrated at constant spinning speed of the disk without any kind of controlling such as surface modification of chip materials, and spinning speed change. This paper describes two different types of autonomous multi step flow sequencing on a centrifugal microfluidic device based on the concept.

DESIGN

Figure 1 shows schematic illustration of timer controlled multistep flow sequencer and flow sequencing on the device. The basic concept of flow sequencing is previously reported. [11] We applied same concept for the design of multi step flow sequencing. In brief, multiple reservoirs for liquid storage is patterned on the PDMS fluidics. The each reservoirs are connected to a clepsydra. When the device is spun, the liquids will be driven toward the outside of the disk due to pressure induced by centrifugal force, while liquid sustain in the reservoir until the flow injection is triggered by the clepsydra. Based on this concept, the system requires only a single steadily spinning motor with a simply fabricated device as the patterned microchannel for flow sequencing.

EXPERIMENT

Polydimethylsiloxane (PDMS; Silpot 184) is purchased from Toray dowcorning. Commercially available blank CD media is used as a substrate of the device. To obtain clear transparent disk, coating film was peeled off as follow. The coating film of the CD was cut with knife and the coating film was peeled by adhesive tape. Then the disk was cleaned using ethanol followed by rinse with water. Finally white adhesive tape is pasted on the surface of CD for better visibility of observation with strobe scope. Thick negative photoresist (SU-8) and SU-8 developer was purchased from Nipponkayaku Co., Ltd.. 4 inch silicon wafer was used for the prototyping of SU-8 mold. The pattern of the device was designed using Auto CAD and converted into Adobe Illustrator file. Then the data was printed on the transparent film with commercial high resolution printing service.

A microfluidic device made of PDMS, 3 mm in thickness is fabricated by the conventional method. [12] First of all, the SU-8 mold pattern is fabricated on the silicon wafer as follows. 4 inch silicon wafers were hard baked on a hot plate more than 5 min at 200 °C and SU-8 (3050) is spincoated to obtain 100 um thick photoresist film. Then the substrate is baked for 1 min at 65 °C and 45 min at 95 °C to obtain dry film. Then the film of SU-8 is exposed to UV light using prepared photomask for 45 sec. The exposed substrate is baked for 1 min at 65 °C and 5 min at 95 °C for the polymerization of exposed part. The baked substrate is immersed into SU-8 developer and rinsed isopropylalcohol finally dried with gentle nitrogen stream. PDMS monomer and catalyst were mixed with ratio of 10:1 in mass and pored on the SU-8 mold and baked at 75 °C for 1.5 hr. The polymerized PDMS film (3 mm in thickness) was peeled form the substrate and cut along the designed shape with knife. The liquid reservoirs and air vent holes were punched and cut with puncher and knives.

The experiments are initialized as follows. The PDMS chips were cleaned with ethanol and water then they are left few tens minutes for the complete drying. The PDMS chips are attached onto the CD by utilizing self adhesion of PDMS onto the disk. The maximum rate that can be applied in the experiment is 3000 rpm. Then all liquid sample (20 ul in volume) and liquid for clepsydra (60 ul in volume) are injected into the reservoirs. All reservoirs are sealed with adhesive tape. The device is placed in a custom-made spinner and spun at a constant rate of 2000 rpm. The acceleration of the spinning is carried out at 1500 rpm/s.

RESULTS

Figure 3 shows experimentally demonstrated multistep flow sequencing. While small volume of liquid leak is observed, however sequential flow triggering is successfully demonstrated and four-step flow sequencing is implemented. Figure 4 shows a different kind of sequencer structure. The five reservoirs are connected like chain with venting channels and chain flow sequencing is implemented from right hand reservoir. The all sequencing is implemented under steadily spinning condition at 1500 rpm, and no additional trigger is applied. Therefore the sequencer is independent from external system by using internal trigger concept. In case of using clepsydra, the timing of liquid injection is determined by the position which the venting channel to be connected on the clepsydra, therefore interval of the liquid injection can be designed flexibly, that means two flow injection can be implemented with small interval, even at the same time, or large interval. On the other hand, in case of chain structure, each injection step must be implemented after completion of each flow injection, therefore series of flow sequencing implemented step by step.

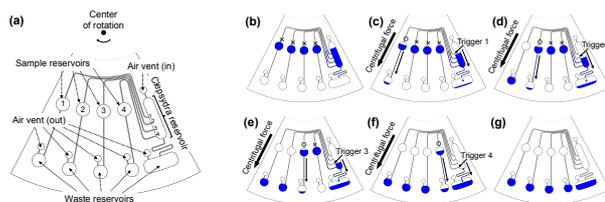


Figure 1: (a) Schematic illustration of flow sequencer. (b-g) Schematic illustration of multistep flow sequencing using internal trigger by clepsydra.

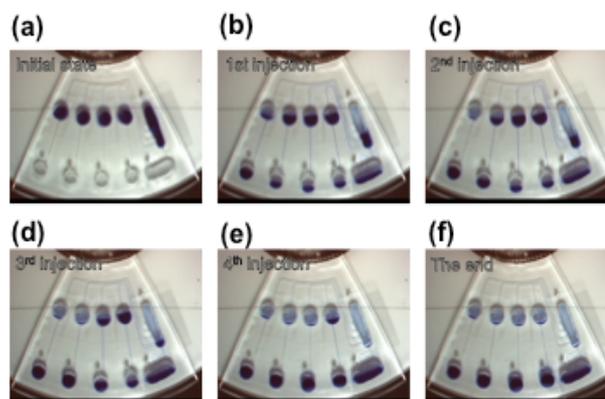


Figure 3. Experimentally demonstrated multistep flow sequencing using clepsydra.

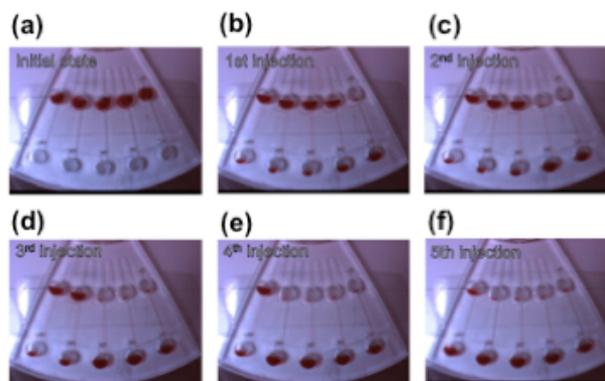


Figure 4. Experimentally demonstrated multistep chain flow sequencing.

CONCLUSIONS

In this paper, we report the demonstration of autonomous multistep sequential flow controls. One is timer controlled sequencing and another is chain flow control. The all sequencing is implemented in few tens of seconds therefore it is necessary to scale up the flow sequencing time up to few tens of minutes to apply more practical chemical processes.

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