Implementation of Femtosecond Laser on the Valve Fabrication of CD-ELISA Process

Samuel E En Lin, and Ya-Fu Chuang

***Abstract*—The burst frequency of designed values are controlled by microfluidic channel width, depth, contact angle of fluid, etc. Among all parameters, geometric factors play an important role in burst frequency. In this study, we utilized a femtosecond laser to develop a "well-type" valve which provides flexibility and on-side fabrication capability of the CD-ELISA process. The developed process and shape can totally eliminated constrain of existing patented design ad use as a "custom-design" work. The proposed "circle-shape" ablation process has shown a good surface control in the "well-type" valve. The works is not limited on the type of material (such as PMMA), therefore, can be used widely in the research stage. For example, it can be served as a single-channel ELISA process using a glass-substrate.**

***Index Terms*—CD-ELISA, Femtosecond laser, Valve, Microfluidic. Biochip**

I. Introduction

Enzyme-linked immunosorbent assays (ELISA), one of the most common immunoassays, is widely used for detection and quantification of chemical and biological molecules (antigens; mainly proteins and polypeptides) and is becoming more and more important in clinical diagnostics, food safety testing, and environmental monitoring [1,2].

A major challenge in developing the proposed CD-ELISA is to control the delivery or flow sequencing of various bio-reagents on the microchips. In a CD microfluidic platform, the centrifugal force provides the pumping pressure. It is important for a CD microfluidic platform to be able to deliver the solution from each reservoir in a pre-specified manner. The delivery of solution from a single reservoir allows the measuring reservoir to be filled without other reservoirs releasing their respective solutions. Capillary burst valves (developed by Gamera, now Tecan) are often used in microfluidic platforms for this purpose [3,4]. Due to protein adsorption, the surface of the capillary valve gradually becomes hydrophilic and the solution wicks through, leading to the failure of the valving function.

Most analytical functions required for a lab-on-a-disc, including metering, dilution, mixing, calibration, and separation have all been demonstrated in the laboratory.

Samuel I En Lin is with the department of Power Mechanical Engineering, National Formosa University, Hu-Wei, Taiwan, ROC(corresponding author to provide phone: 886-6315424; fax: 886-56312110; e-mail: samlin@ nfu.edu.tw).

Ya-Fu Chuang is with Femtosecond Laser Technology Department, Laser Application Technology Center, Industrial Technology Research Institute, ITRI South, Taiwan.

.
This kinetic increase of the hydrophilicity of the polymer has an effect on the performance of the capillary valve. As a liquid flows through a sudden expansion, asymmetric intermolecular forces at the liquid-air interface generate an opposing surface tension force. If the net capillary pressure due to the surface tension force is greater than the net centrifugal pumping pressure, the capillary valve will hold the liquid [4]. However, the magnitude of the surface tension force, and hence the opposing capillary pressure, is very sensitive to the magnitude of the contact angle. Thus, a capillary valve that initially holds a flowing fluid could fail over time as protein adsorption renders the surface increasingly hydrophilic, decreasing the contact angle and hence the opposing capillary pressure.

A novel "well-type" capillary valve has been developed that seeks to overcome these problems. The valve contains a series of holes (e.g. two or three wells) which is arranged in a series. If the first well fails within the entire well valve due to protein adsorption, the fluid will flow to the second well, and then to the third, etc. These well-type valves provide the necessary redundancies to hold the fluid for a prolonged period of time when protein adsorption causes premature valve failure.

II. Design and Fabrication Method

A. Valve design and analysis

In the CD microfluidic platform, the centrifugal force provides the pumping pressure. The capillary burst valve is a passive valve that requires no moving parts. It is controlled by the angular speed of rotation, fluid density, surface tension, and geometry and location of the channels and reservoirs. The bust frequency can be expressed as [5],

$$f_{bn} = \left[\left(\frac{\gamma}{4\pi^2 \rho \left(R_2 - R_1 + (n-1)(d+\omega_f)\right) \left(\frac{R_1 + R_2 + (n-1)(d+\omega_f)}{2} \right)} \right) \left(\frac{2 \cdot \sin\theta}{\omega_c} - \frac{\cos\phi_{Top}}{h_c} - \frac{\cos\phi_{Bot}}{h_c} \right) \right]^{1/2} \quad (1)$$

The related notations are listed in Table 1. With the current design parameters, we are able to compute the theoretical burst frequency. The proposed design of well-type valves is sketched in Fig.1 with three loading holes and three reservoirs as illustration.

B. Experimental setup

Our experiments used Ti: Sapphire femtosecond laser system which had a central wavelength of 800nm, a couple duration of 120fs and pulse repetition frequency is 1KHz. Fig.2 contains a detailed diagram of the beam optical system. The pulse energy from the system, 2W was attenuated to the range of from 10mW to 500mW by thin-film polarizing beam splitter (PBS) and a half-wave plate. The initial beam output diameter is 9.6mm and quality parameter (M^2) is 1.3. There is a 10X microscope objective (M Plan Apo NIR, Mitutoyo) used in this experiment. The NA of the 10X objective was 0.26. The focus spot radius can be calculated by

$$\omega_0 = \frac{1}{\pi} M^2 \frac{\lambda}{NA} \tag{2}$$

The propagation of higher order Gaussian beam which includes quality factor can be expressed by

$$\omega(z) = \omega_0 \sqrt{1 + \left(\frac{M^2 \lambda z}{\pi \omega_0^2} \right)^2} \tag{3}$$

where M^2 is the beam quality factor, z is the propagation distance from the focus location, and λ is the wavelength. The relation between spot radius and resultant ablation hold radius under different pulse energy is detailed in the next section.

The experimental setup for microfluidic testing is shown in Figure 3. The disc is mounted on a motor plate (up to 5000 rpm) designed by our lab. The working fluid is DI water and it was loaded by a hand-pipette. Up to the reservoir filled up to 90%, we stopped the loading process. The motor control is constructed around a five-stage stepper motor, a 12-V power supply, and a VB controlled interface. It allows accurate monitoring of both position and acceleration/deceleration profiles as the motor is running.

III. RESULTS

The femtosecond laser was control by motion stage and the process parameters. The channel surface quality can be monitor by surface profilometer (polytec TMS6X0) ar NFU facility. The 50X microscope objective was used in order to measure the ~10um channel quality. We applied 300mW and can achieve surface roughness Ra around 200~300n in the fabrication. The molded CD microfluidic channel has Ra value of 200nm while Ra~300nm can be achieved using laser ablation with a proper ablation path and applied power. The three dimension fabricated well-type valve is shown in Fig. 4. Reducing the channel and valve size can greatly has time saving impact on the ELISA reaction zone [4].

Demonstration of holding time and burst frequency on the well-type valve is plotted in Fig. 5. The theoretical computation is based on the values listed in Table 1. In order to perform the experiment, we used scotch tape as channel lamination. Both experiment and theoretical show a good agreement as indicated in Fig.5. The overall hold time of a capillary fishbone valve is simply the sum of the individual hold times of each fishbone within the valve. In this case, the holding time is about 15 min for the proposed three-well design.

IV. CONCLUSIONS

Experimental work and mathematical modeling have been applied to quantitatively understand the behavior of the novel well-type valve. The femtosecond laser was applied in the valve fabrication and has demonstrated to have a good success on the holding time control. The advantage of using femetosecond laser is on the precision well control and also on the independence of substrate material. The roughness of laser ablation can be control within Ra~300nm range and it is workable on the biological applications.

ACKNOWLEDGMENT

The authors would like to thank James L. Lee at OSU, USA for his invaluable assistance in this research. Also the authors would like to thank the Ministry of Education, Taiwn, for the funding support and NFU/RSH for the equipment usage.

REFERENCES

[1] Madou, M. , Lee, L., Daunert, S., Lai., S., Shih, C., “Design and Fabrication of CD-like Microfluidic Platforms for Diagnostics: Microfluidic Functions, Biomedical Microdevices, 2001, 3:3, 245-254.
[2] Lee, L., Madou, M., Koelling, K., Daunert, S., Lai, S., Koh, C., Juang, Y., Lu, Y.,Yu, L., “Design and Fabrication of CD-like Microfluidic Platforms for Diagnostics: Polymer-Based Microfabrication,” Biomedical Devices, 2001, 3:4, 339-351.
[3] Lai, S., Wang, S., Luo, J., Lee, L., Yang, S., Madou, M., “Design of a Compact Disk-like Microfluidic Platform for Enzyme-Linked Immunosorbent Assay,”Anal. Chem., 2004, 75, 1832-1837.
[4] Feng, Y., Zhou, Z., Ye, Xiongying, Y., Xiong, J., “Passive Valves Based on Hydrophobic Microfluidics,” Sensors and Actuators, 2003, 108, 138 -143.
[5] Samuel Lin and RS Chen, Parametric Study on the Burst Frequencies of Fishbone Capillary Valve used in the Microfluidic CD ELISA, The 15th National Computational Fluid Dynamics Conference, 2008 Aug.15, Taipei, Taiwan.

Table 1: Definition of variables

Variable	Definition	Values used in Simulation
w_c	Width of microchannel	300um
h_c	Depth of microchannel	300um
w_f	Width of fishbone valve	500um
d	Distance between fishbones within a fishbone valve	
n	Number of fishbones within a fishbone valve	3
R_1	Distance from CD center to beginning of fluid reservoir	21.98mm
R_2	Distance from CD center to end of fluid flow front	25.92mm
ρ	Fluid density	1
γ	Air-liquid surface tension	72.9
θ	Top-view contact angle (width direction)	50~60
ϕ_{Bot}	Side-view contact angle on bottom of channel (height direction)	50~60
ϕ_{Top}	Side-view contact angle on top of channel (height direction)	60~80
f	Actual spin frequency of the CD	TBA
f_{bn}	Burst frequency of nth fishbone in fishbone valve	TBA

$F_{h,top}$	Surface tension force vector on top of channel from side-view (height direction)	
$F_{x,h,top}$	X-direction surface tension force vector on top of channel from side-view (height direction)	
$F_{h,bot}$	Surface tension force vector on bottom of channel from side-view (height direction)	
$F_{x,h,bot}$	X-direction surface tension force vector on bottom of channel from side-view (height direction)	
F_w	Surface tension force vector on one wall from top-view (width direction)	
$F_{x,w}$	X-direction surface tension vector on one wall from top-view (width direction)	

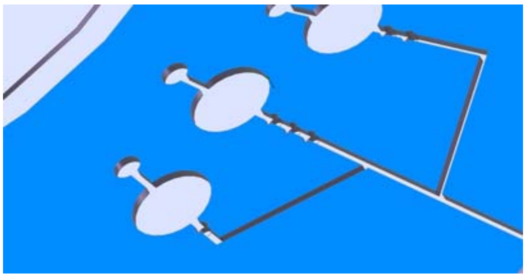

Fig.1: Sketch of the proposed well-type valves. The first zone is loading hole and reservoir is the next, then it is the well-type valves.

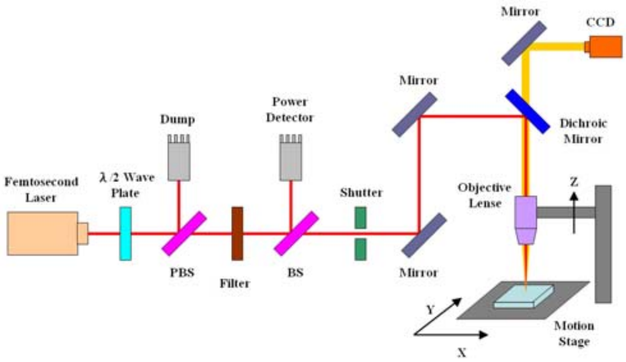

Fig.2: The experiemntal setup of femtosecond laser. The CD-ELISA is lcoated on the motion stage. Three axes can be moved using NC programing.

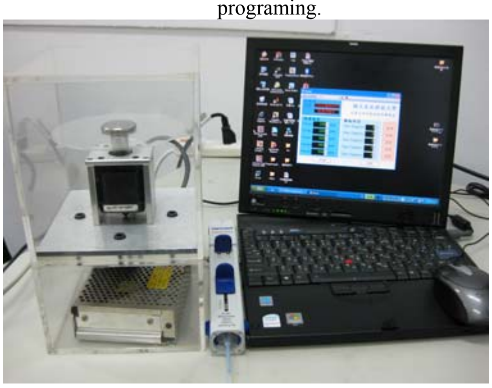

Fig.3: Schematic of Experimental Setup for Burst Frequency Measurements. The computer can control the motor RPM in different connectted prifile

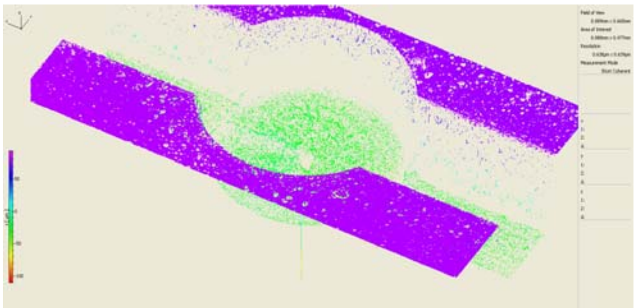

Fig. 4: The 3D well-type valve is fabricated by the femtosecond laser. The hole is about 50um and it is used in a 25um micro channel.

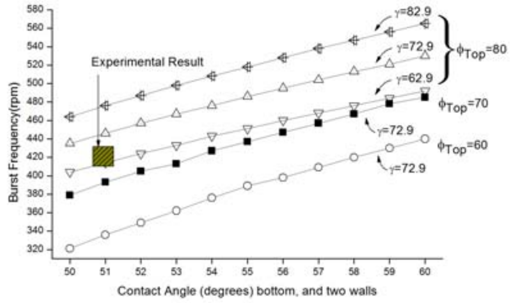

Fig. 5: The comparison of theoretical and experimental burst frequency. Contact angles inside channel surfaces, such as side and bottom walls, covered scotch tape, are measured by separated equipment.