

# An Chip-based Instant Protein Micro Array Formation and Detection System

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## ABSTRACT

This paper proposes a novel protein micro arrayer employing 3-in-1 chip system, instantly delivering hundreds of biosamples in parallel for disease diagnosis. The 3-in-1 chip system included: micro filling chip, micro stamper array and micro bio-reaction chip, applicable to high throughput disease diagnosis and drug screening. Capillary force is applied to drive liquids flowing through all the chip channel system. The filling chip contains hand-fill-in reservoir and sealed with PDMS thin film for protein storage and loading to arrayer. The uniformity of the printed spots by the stamper array can be within 10% standard deviation. The micro photo sensor array is designed for the fluorescent detection. Two human cancer (HURP and E6) markers have been successfully detected by this micro stamper system. Reproducibility and Surface wettability for micro/nano droplet formation around stamping process are investigated by simulation and experiment in this paper.

## 1 INTRODUCTION

The development and application of protein arrays have become an important trend in modern biomedical diagnoses because of micro-array's ability of massive parallel process [1]. Protein micro arrays have become powerful tools in biochemistry and molecular biology. Array-based technology involves two principal processes: transferring hundreds of biosamples onto substrates, and immobilizing the biosamples on the substrates. "Micro contact printing" [1-4] is one of the microarray technology to simultaneously and rapidly imprint a vast number of bio-reagents in a short time. The conventional way to produce micro arrays utilized a needle array and computerized robot system to select and spot numerous bio reagents [3]. This arraying system has the drawbacks of high cost, large size variation, and serial and long spotting process, which may malfunction proteins from dry-out. As a result, "Micro contact printing" [1-4] has been becoming an emerging technology to simultaneously and rapidly imprint a vast number of bio-reagents in a short time. Among various micro printing schemes, a new type microarrayer has been proposed by us recently [5] by employing micro stamping method to simultaneously immobilize hundreds of proteins in parallel. However, because of the short distance among stamping heads, it becomes necessary to employ a filler chip[6] for protein

fill-in in batch instead of fill in individually. The combination of the batch fill-in and batch arraying process enable a rapid and high throughput formation of protein array to prevent proteins from de-naturization during the array process.

Besides the batch fill-in, parallel detection of protein array is also important for rapid and high throughput diagnosis/screening. Among many detection methods, optical detection is one of the most applied detection means because of its high sensitivity and reliability. As a result, the proposed system employ micro optical system to directly couple weak fluorescent light emitted from protein arrays through micro optical collectors onto the micro sized photodiode, thus greatly improves the efficiency of light detection by reducing the light path from meter range to sub-millimeter range.

By employing the three-in-one chip system as mentioned above, parallel protein array formation and detection process can be realized, as shown in Fig. 1. Proteins can be loaded and preserved as a library in each fill-in reservoir in the micro filling chip before real application. When protein diagnosis/screen desired, the micro array stamper can be filled in proteins in parallel from the filling chip by piercing the needle array on the top of the stamper into the membrane of the filling chip at once. Protein array can then be formed simultaneously by the gentle contact between the stamper and bio reaction chip. The formed protein array is now ready for parallel bio reaction. Micro optical detection system is setup on the bottom of the bioreaction chip for parallel protein detection. Thus high throughput protein diagnosis/screen process can be finished in hours including bioreaction

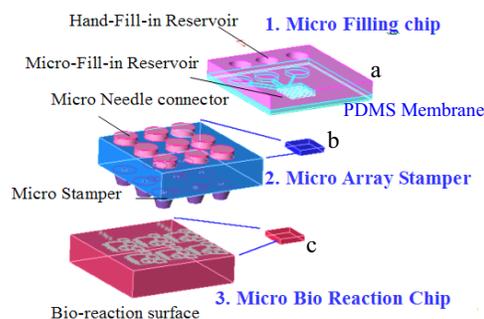


Fig1 integration of chip system

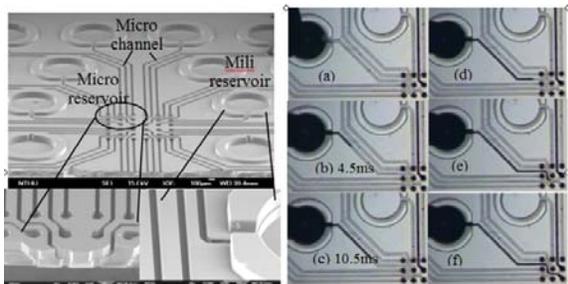


Fig2. micro filling chip and filling test

process for hundreds to thousands of proteins at one time. The detail operation and design of the 3-in-1 chip system is elaborated in the following sections.

## 2 BIOCHIP SYSTEM DESIGN

### 2.1 Micro Filling Chip

The operational concept of protein filler chip to perform batch fill-in process for micro arrayer is shown in Fig. 1a. After we dispensing different proteins in the filler chip, parallel protein fill-in process can be carried out by directly contact the filler chip with the arrayer chip, thus tens to hundreds of proteins can be transferred by capillary force from the filler into the arrayer chip in seconds. This process can be repeated to fill another arrayer chip until the empty of the filler chip. The Filler chip is not only employed for protein filling purpose, but also applied to preserve the stored protein library permanently by sealing the top and bottom surface with PDMS thin film until the need for arrayer feeding.

The filler chip contains mili-reservoirs on the chip top for protein sample dispensing, micro reservoirs on the bottom for holding protein solutions, and micro channels connecting each mili-reservoir to the corresponding micro reservoir. Protein samples can be filled-in by capillary force from individual mili-reservoir to the corresponding micro-reservoir automatically without external power. To fill in protein solutions vertically and allow proteins arranged on a two-dimensional array, three-dimensional fluidic structures are desired. The fabricated filling chip and the loading process is shown in Fig. 2.

### 2.2 Micro Stamper Chip

As shown in Fig. 1b, the stamper chip can be used to spot different kinds of biosamples simultaneously

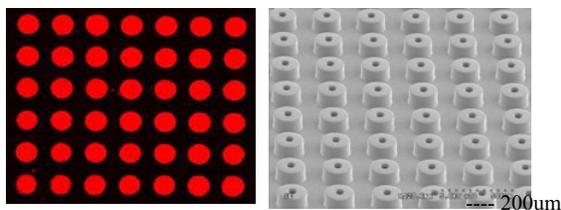


Fig3 Fluorescent stamp result and uniform PDMS micro stamper array

and expanded to stamp hundreds of different biosamples. The micro stamper comprises micro-needles, micro-stamp array, and a microchannel array connecting the reservoirs and micro-stamp array. Different proteins could be dispensed into different channels and driven by capillary force to the tips of the micro stamps through the micro channels. The protein-filled micro stamper was then brought into contact with the bio-assay chip to generate sample arrays for further bio processing. Elastomeric polydimethylsiloxane (PDMS) is used to fabricate the micro stamps, so conformal contact can be achieved on rough surfaces. The uniform fluorescent spot of stamping result and the picture of the PDMS stamper was shown in the Fig3.

### 2.3 Micro Bio-Reaction Chip

After the proteins were delivered to the bio-assay chip, the binding strength between the bio-assay chip and proteins is important for subsequent bio processing. Protein immobilization means are categorized into three major types: carrier-binding, lattice-type, and micro-capsule-type immobilization. Among those three, carrier-binding is more favorable for protein chip application for its properties of thin molecule film and ease of optical or electrochemical detection. Covalent bond is the strongest bond of carrier-binding. As a result, covalent bond of proteins by SAMs to carriers (glass slide in this study) is chosen for the application of our micro stamping system.

Under the immobilized protein array, micro optical system with APD (avalanche photo diode) sensor arrays are employed for fluorescent detection. APD sensor made by the conventional CMOS process was easy to be integrated with micro array. The avalanche effect under a suitable reverse voltage produces an internal-gain to amplify the weak fluorescent signal. As a result, an integrated micro optical system to detect weak fluorescent signals is designed underneath the micro reaction chip. The conceptual design of the sensor array and the fabricated sensors is shown in Fig. 4.

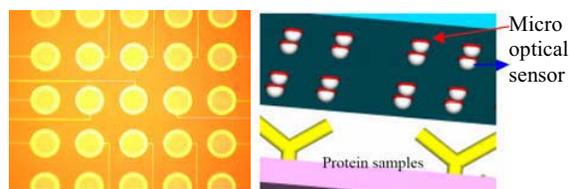
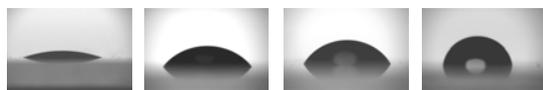


Fig4 (a) Optical microscope picture of APD photo diode sensor (b) the illustration of the photo sensor detection

## 3 MATERIAL

The surface properties of bioreaction chip greatly affect the stamped droplet size. Thus, surfaces with different wetting properties were prepared by glass slide, APTS (aminopropyltrimethoxysilane) and BS<sup>3</sup> (bis-sulfo-

succinimidyl suberate) on slide, APTS and DSC (N, N'-disuccinimidyl carbonate) on slide, and Su8 photo resist (MicroChem) coating on slide. The surface peak roughness is ranged from 73.8nm to 148nm. The deformation of the elastic stamper, which is made of PDMS, can make a conformal contacting with the substrate. The effect of surface roughness to the stamped feature size can be ignored. The hydrophilic properties of those surfaces have been characterized and illustrated in Figure 5, ranging from 18° to 78°, respectively on different surface coating. PBS buffer with 30% glycerol and 1% Cy3 dye is used as the stamping fluid in experiment. Both fluorescent and optical microscope images are used to measure the stamping feature size.



(a)18° (b)51° (c)58° (d)78°  
 Figure 5. The contact angle of the PBS solution with 30% glycerol on different substrate surface (a) Glass (b) APTS+BS<sup>3</sup> coating (c) APTS+DSC coating (d) Su8 photo resist coating

#### 4 SIMULATION AND EXPERIMENT

To fully understand the flow behaviors of the stamping system, numerical methods are employed to simulate the stamping process for effective design through solving first principle equation. Governing equations as conservation laws of mass and momentum describing both liquid and gas phases are included. Most of all, surface tension force is the major driving force and the total surface force depends on the orientation and surface area of the liquid-gas interface, precise determination of the moving location and shape of the interface is the key issue of accurate computation. Numerical models [7] consist of Volume-of-Fluid (VOF) method for two-phase homogenous flow model and the interface tracking technique in cooperation with CSF Continuum Surface tension Force model. In order to discover the dynamic stamping process and the drop breakdown process, 3D simulation was demonstrated as shown on the Figure6.

#### 5 RESULTS

The high-speed camera under the transparent glass substrate sequences the droplet formation and the separation of the micro-stamper. The dynamic spot size varying with the time can be analyzed and compared with the 3-D dynamic simulation. Meanwhile, the stamped spot size varies with the different substrate wettability as shown in the Figure7. On the Su8 coating substrate, the printed spot size is 47μm, which is much smaller than that on the glass substrate of 123μm.

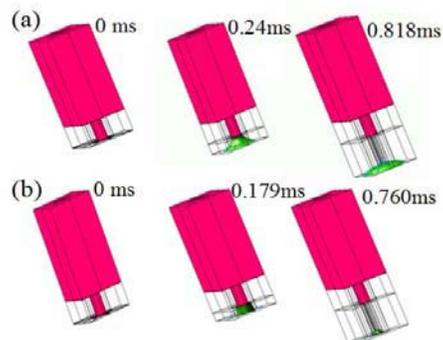


Figure 6. Dynamic simulation of stamping process (a) on higher hydrophilic surface (b) on lower hydrophilic surface

For the minimum spot size of the conventional quill pen microarrayer is 75μm, the physical property of the printing liquid and substrate has to be precisely controlled. Features containing fluorescent dye are printed as shown on the Figure7. The spot size increases with substrate wettability, which is observed in both simulation results and experiments as illustrated in Figure8. The PBS solution plus 30% glycerol (viscosity 3.20cp) makes the feature size smaller than that of the BSA (bovine serum albumin) protein solution (viscosity 1.02cp), and the simulation result for the former solution agrees well with the experiment one. It demonstrated that the wettability of the substrate surfaces and the viscosity of stamping solution could control the stamping spot size of the micro stamping process.

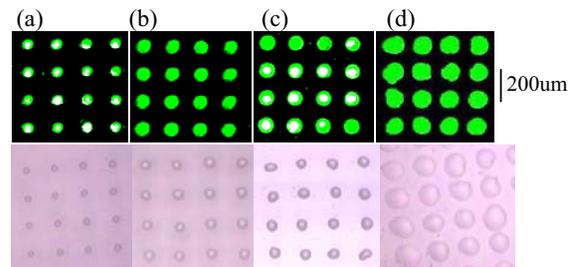


Figure 7. Drop features on different substrate observed from optical microscope and fluorescent image (a)Su8 photo resist (b) APTS+DSC (c) APTS+BS<sup>3</sup> (d) Glass

Fig9 shows the stamping results, and demonstrates a repeatable stamping within a deviation 10% in size and 25% in intensity. In the repeatability testing of the micro stamping process, the micro stamper arrayer was filled with 2 kinds of fluorescent solution. One is cy3 0.001 μg/ μl with PBS solution and glycerol 30% and the other is cy5 0.1 μg/ μl with PBS solution and glycerol 30%. It is 12 times that the micro stamper was pressed

continuously on the substrate without cross contamination as shown on the Fig10. The glass substrate is coated by APTS+BS<sup>3</sup>.

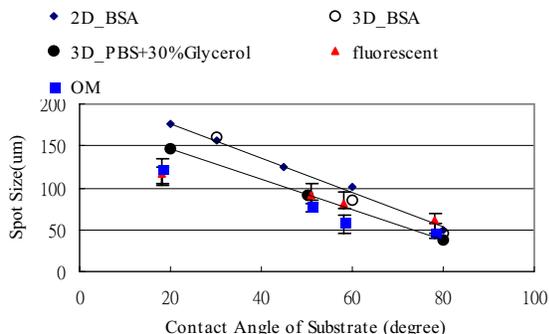


Fig 8. Spot size varied with wettability of substrate surface observed in simulation results and experiments.

In the testing of utility of protein stamper for cancer marker detection, four kinds of solution of the tumor marker antigen (HURP: liver cancer, E6: Papillomavirus antigen) are filled into the micro stamper and printed to transfer the protein on the substrate. Through the ELISA testing, the antigens with different concentration have been recognized as shown on the Fig11 in a concentration of E6 76 ng/µl. It demonstrates that the micro stamper can successfully transfer proteins into array simultaneously for disease detection.

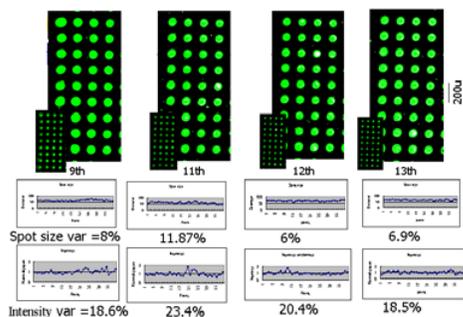


Fig9 . Protein spots uniformity with each stamp result

## 6 CONCLUSIONS

This paper introduces a 3-in-1 chip system for protein array rapid immobilization and detection. The operation and testing of the three chips, including micro filling chip, micro stamper chip, and bioreaction chip has been successfully carried out. The testing result shows the spot size of soft printing decreasing with the lower hydrophilic substrate surface, demonstrated by both simulation and experiment. The test of stamping uniformity and repeatability has been test performed. It demonstrates a repeatable stamping result within a

deviation 10% in size and 25% in intensity. Two human cancer (HURP and E6) markers have been successfully detected by this micro stamper system. The micro stamper array successfully prints the protein to the substrate. It can be applied on the disease detection, diagnosis and drug discovery.

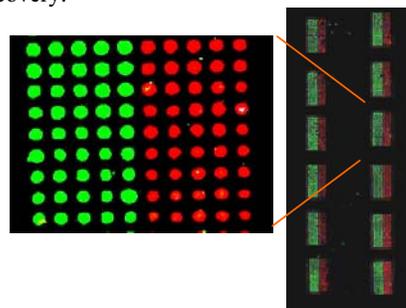


Fig 10 Stamp two types solution cy3 cy5 simultaneously in 12 times.

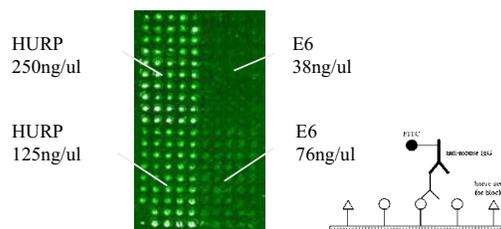


Fig11. ELISA of tumor marker detection. HURP : liver cancer antigen, E6: Papillomavirus antigen

## REFERENCES

- [1] S.C. Lin. et al., "Microsized 2D protein arrays immobilized by micro-stamps and micro-wells for disease diagnosis and drug screening" *Analytical and Bioanalytical Chemistry*, Vol. 371, pp. 202-208, 2001.
- [2] F.G. Tseng, et al., "Protein micro arrays immobilized by  $\mu$ -stamps and protein wells on PhastGel pad" *Sensors and Actuators B*, Vol. 83, p. 22-29, 2002.
- [3] Kane, R.S. et al., "Patterning proteins and cells using soft lithography" *Biomaterials*, Vol. 20, p.2363-2376, 1999.
- [4] F.G. Tseng, et al, "Fluid filling into micro-fabricated reservoirs" *Sensors and Actuators A*, Vol. 97-98, p131-138, 2002
- [5] S.C. Lin, et al., "A novel protein micro stamper with back-filling reservoir for simultaneous immobilization of large protein arrays" *IEEE MEMS*, Kyoto, Japan. p299-302, Jan. 2003
- [6] F.G. Tseng, et al., "Micro protein filler chip for protein library preservation and arrayer filling in batch"  $\mu$ TAS, Squaw Valley CA, USA, p639-643, Oct. 2003
- [7] Brackbill, J.U. et al., "A continuum method for modeling surface tension" *Journal of computational physics* Vol 100, p335-354, 1992