# IMMUNOSENSING USING FABRICATED PROTEIN MICROARRAYS

S.W. Howell*, H.D. Inerowicz**, L. Guirl*, F. Regnier**, R. Reifenberger*

*Dept. of Physics, Purdue University, West Lafayette, IN, USA, howell@physics.purdue.edu
**Dept. of Chemistry, Purdue University, West Lafayette, IN, USA, inerowic@purdue.edu

## ABSTRACT

Patterned microarrays of antibodies were fabricated and tested for their ability to bind targeted proteins and bacteria. These arrays were used for a series of protein and bacterial immunoassays to detect Immunoglobulin (IgG) proteins, *Escherichia coli* 0157:H7 and *Renibacterium salmoninarum* (RS) bacteria. The microarrays were fabricated using microcontact printing (μCP) and microfluidic techniques. Characterization of the microarrays was conducted by scanning probe microscopy (SPM) and fluorescence microscopy (FM). The high-resolution SPM imaging showed that targeted bacteria and proteins had a higher binding selectivity to complementary antibody patterns than to non-functionalized regions of the substrate. These studies demonstrate how protein microarrays could be developed into useful platforms for sensing microorganisms.

***Keywords***: SPM, Immunosensor, Bacterial Detection, Microcontact Printing, Microfluidic.

## 1 INTRODUCTION

Microarray technology is evolving from genomics to proteomics. Protein arrays will be useful in miniaturization of biosensors, clinical immunological assays, and a series of basic research applications ranging from mapping the proteome to examining protein:protein interactions.

By patterning specific sensing proteins onto well-characterized substrates, the surface of the substrate becomes functionalized to bind complementary proteins. Over the past decade, several techniques (microcontact printing (μCP) and microfluidic flow channels) have been developed to pattern molecules on surfaces. We have demonstrated that these techniques can be used to economically fabricate protein arrays with sub-micrometer precision. Unlike DNA microarrays, the technology for generating protein microarrays is still developing. The micropatterning of protein arrays are of great interest today for this reason [1]. The low cost of fabrication compared to lithography, as well as simplicity of transferring proteins without loss of biological activity, makes theses methods very attractive.

μCP stamps and fluidic channels were used to fabricate biologically active substrates capable of performing multiple immunoassays. Subsequent SPM characterization revealed that individual array elements have a high level of homogeneity. Cross-reaction studies between non-specific proteins revealed that protein arrays show low non-specific binding of non-targeted compounds while preserving the high affinity of antibodies to bind antigens.

## 2 MICROARRAY FABRICATION

Polydimethysiloxane (PDMS) elastomer has been found to be a good material for the manufacturing of μCP stamps and microfluidic channels. μCP stamps can be fabricated by molding the PDMS on a silicon master (Figure 1a). After appropriate surface treatment, a micropatterned PDMS stamp provides an excellent material for the direct transfer of adsorbed proteins to an inorganic substrate. Similarly, PDMS microfluidic channels (Figure 1b) are an easy way to deposit patterned proteins on substrates such as glass, silica or gold [2].

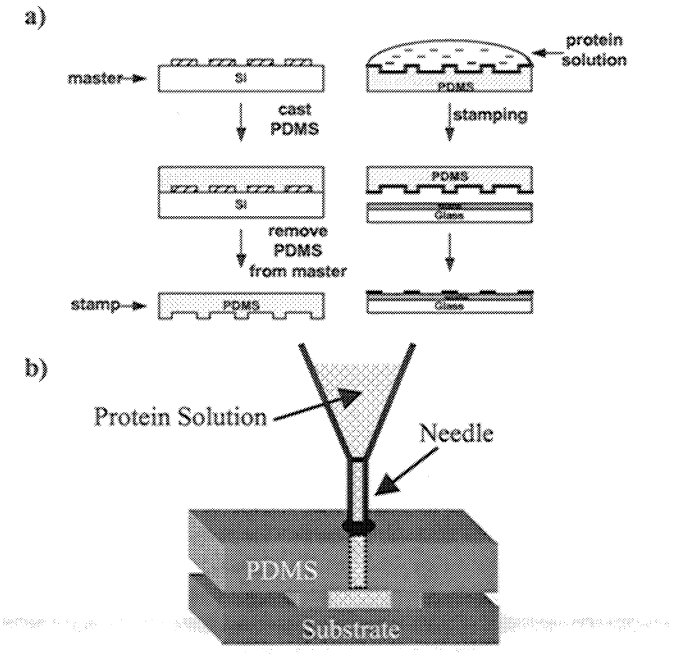



Figure 1: Schematic diagrams illustrating a) the μCP technique b) the microfluidic technique of patterning proteins onto substrates.

To demonstrate a capability of identifying two different proteins in solution on a single substrate, a PDMS stamp was used to deposit a micro-square pattern of mouse IgG on a functionalized substrate [3]. Next, the substrate

was immersed in a solution of human IgG to backfill the substrate between the patterned squares of mouse IgG. Since this substrate now has two different sensing proteins immobilized on the surface, a distinctive change in the surface morphology will develop when the substrate is exposed to complementary proteins such as anti-human or anti-mouse (see Figure 2). A similar process was followed to prepare a substrate to detect targeted bacteria.

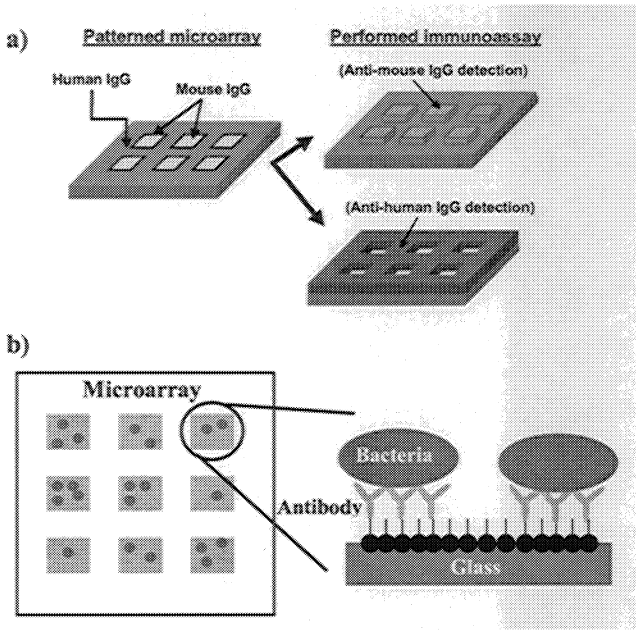



Figure 2: Schematic diagrams of immunoassays for detection of targeted proteins and bacteria.

## 3 CHARACTERIZATION AND DETECTION

Precise verification of the quality of printing and the efficiency of the complimentary antibody binding were obtained using a commercial scanning probe microscope. In addition to SPM characterization, fluorescence microscopy was used as a diagnostic to verify performed immunoassays of various IgG proteins [3].

Figure 3a shows a false-colored topographic SPM image (acquired in contact mode) of the substrate after exposure to anti-human IgG. The human:anti-human IgG coated regions (regions between the squares) were found to be 3 ± 2 nm higher (brighter in the SPM image) than the regions containing only mouse IgG (squares). This verifies that a specific binding occurred only in the targeted regions.

The SPM image shows numerous objects located in the mouse IgG regions. These objects have heights that are 3 to 5 times larger than the regions containing the human:anti-human IgG. There were also objects with dimensions on the order as the anti-human IgG. These could be regions of cross-reaction. Unfortunately, the SPM could not determine the nature of these objects. However, the anti-human IgG was labeled with a fluorescence tag. Therefore, if these objects were residues related to the anti-human:mouse IgG cross-reaction, they might be detected by fluorescence microscopy.

Figure 3b is a fluorescence microscopy image of the same sample studied by SPM. As expected, the image shows fluorescence in the regions between the squares containing human:anti-human IgG, confirming the selective binding of the target protein. This result, in addition to the SPM data, shows that a targeted protein interaction has occurred. Careful examination of the fluorescence microscopy images provides evidence that the residues observed in the SPM images are related to cross-reaction between anti-human and mouse IgG. From these images we estimate that 10-15 % of the surface is affected by unwanted cross-reaction products.

Similarly fabricated substrates were also exposed to solutions of anti-mouse IgG. SPM characterization of these substrates revealed a change of morphology in the square regions, indicating the presence of anti-mouse IgG bound to the mouse IgG sensing layer.

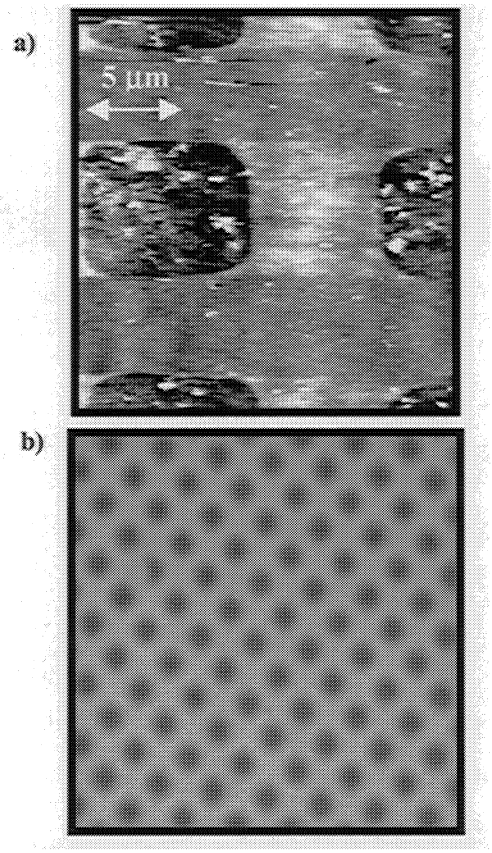



Figure 3: a) SPM and b) FM images of substrate after exposure to anti-human IgG (region between squares)

To extend this methodology to bacterial species, antibodies to targeted bacteria were patterned on glass substrates [4]. Figures 4a and b show false-colored non-contact SPM topographic images of the immobilized antibodies to both the *E. coli* before and after performing a bacterial assay. These images show the presence of large

objects, with an average height of 130 ± 40 nm. Most importantly, these objects are observed to have a higher binding affinity to the printed antibody regions. Figure 4c shows a similar assay for *Renibacterium Salmoninarum* (RS) bacterium. A grid with a 15 μm pitch is superimposed onto the image to show the periodicity of the bacterial pattern that overlaps closely with the pattern printed onto the substrate.

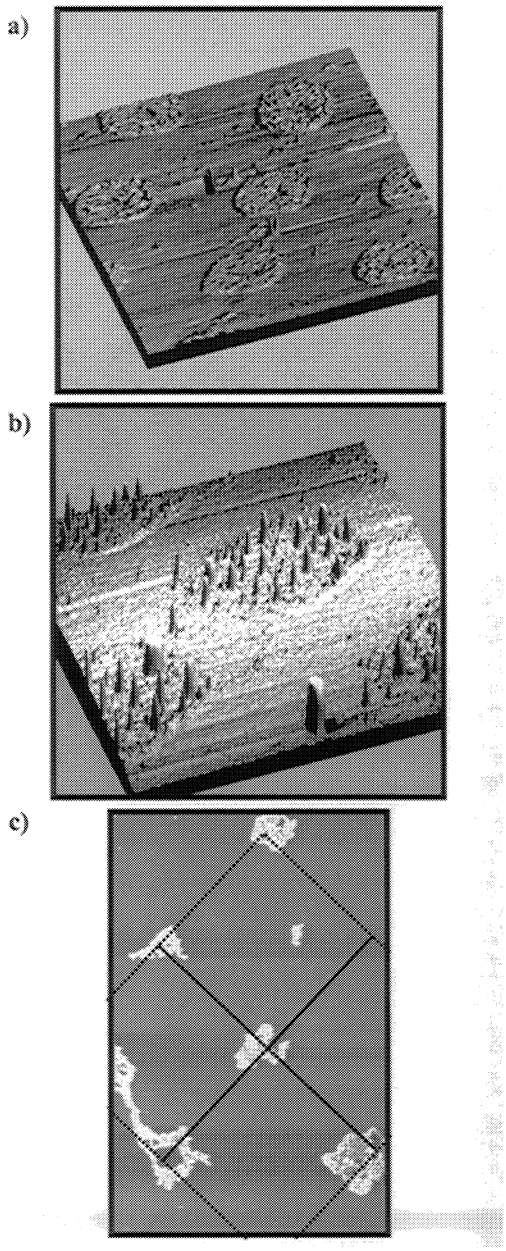



Figure 4: SPM images showing a) the substrate before (30 x 30 μm image) and after incubation with *E. coli* (15 x 15 μm image). In c), an SPM image (50 x 30 μm) of a substrate used to detect RS bacteria.

## 4 MULTI-PROTEIN ASSAYS

More research is underway to understand the necessary requirements to move beyond this initial research demonstration. To develop this simple chip methodology into a practical system, several fundamental parameters like the substrate's shelf-life and sensitivity must be determined. In addition, the fabrication of more complex protein patterns (substrates consisting of more than 2 sensing proteins) must be demonstrated.

To extend our studies for multi-protein detection, we used microfluidic network for deposition of proteins. Our fist approach for fabricating more complex substrates was to utilize the microfluidic network shown in Figure 5. This microfluidic network allows us to pattern four different sensing proteins onto a single functionalized glass substrate. Each of the four segments of the network creates a patterned array of a particular sensing protein, creating multiple sensing regions (Figure 5b).

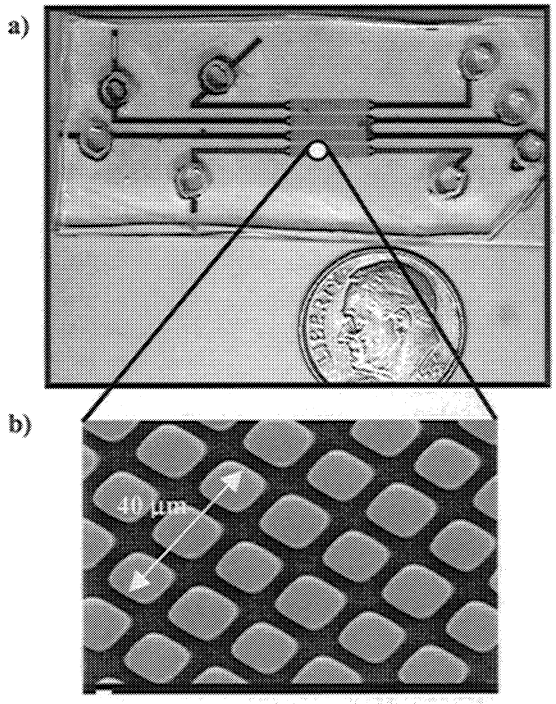
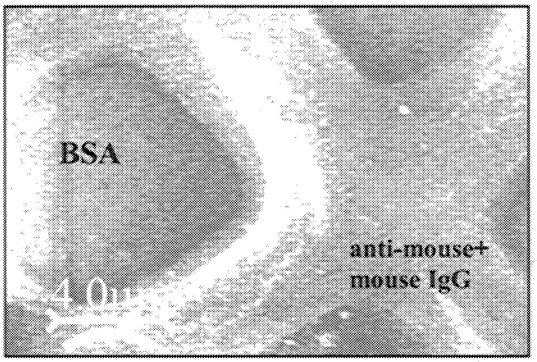



Figure 5 a) Microfluidic network used to immobilize four different sensing proteins onto a functionalized glass substrate. b) Scanning electron microscope image of the PDMS channel.

Each channel of microchip shown (Figure 5) is filled with different antibodies. The various antibodies are adsorbed from the solution flowing through the channels. After 0.5 –1.0 hour, the PDMS chip is pilled off, the substrate is washed and non-patterned areas are blocked by soaking the substrate in a solution of bovine serum albumine (BSA). Prepared in this way, the substrates are now ready to bind complementary proteins and bacteria.

Figure 6 shows the SPM and fluorescence results from one region of the chip after performing multiple immunoassays. In this region, anti-mouse IgG was absorbed onto a sensing layer of mouse IgG that had been deposited onto a functionalized glass substrate. Figure 6a is a non-contact SPM image of this region. The image clearly shows a morphological modification (an increase in height) of the array after binding of anit-mouse to the mouse IgG regions.

a)




b)

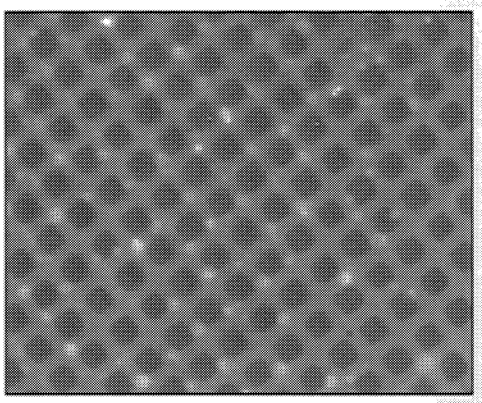


Figure 6. a) SPM and b) FM images of substrate after exposure to anti-mouse IgG (Mouse IgG deposited by microfluidic in 5μm wide channels). The SPM image shows an increase in the height of the array after performing the assay (brighter color indicates increase in topographic height).

Additional experiments were conducted to determine the amount of non-specific binding. Using substrates prepared in a similar manner as mentioned above, regions of the multi-protein chip were exposed to non-complementary IgG proteins. The results of this study showed that non-complementary IgG proteins did not bind to targeted proteins regions. These results were confirmed by both characterization methods.

## 5 CONCLUSION

We describe a simple technique for fabrication of antibody microarrays capable of detecting bacteria and simple proteins. The microarrays were inexpensively fabricated by either μCP or micro-fluidic methods. The fabrication of the arrays was optimized by careful characterization using scanning probe microscope techniques. SPM studies of exposed antibody arrays indicated a high binding specificity of bacteria and proteins to their complementary antibody. The results of the cross-reaction studies show that the bacteria have a low binding efficiency to non-complementary antibodies. Taken all together, these results suggest a promising and inexpensive approach for the detection and rapid identification of targeted proteins and bacteria.

## 6 ACKNOWLEDGEMENTS

The authors gratefully acknowledge support from U.S. Navy grant No. N00164-00-C-0047, The authors would also like to acknowledge the encouragement and many helpful discussions with Prof. Y.E. Kim, the Director of the Center for Sensing Science and Technology at Purdue University, during all phases of this work.

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