# Improvement of Maskless Photolithography of Bio Pattern with Single Crystalline Silicon Micromirror Array

**Yun-Ho Jang*, Kook-Nyung Lee**, Jae-Hyoung Park***, Dong-Sik Shin****, Yoon-Sik Lee**** and Yong-Kweon Kim†**

**Abstract** – This study focuses on the enhancement of maskless photolithography as well as the peptide synthesis application with single crystalline silicon micromirrors. A single crystalline silicon micromirror array has been designed and fabricated in order to improve its application to the peptide synthesis. A micromirror rotates about ± 9° at the pull-in voltage, which can range from 90.7 V to 115.1 V. A 210 μm-by-210 μm micromirror device with 270 μm mirror pitch meets the requirements of an adequately precise separation for peptide synthesis. Synthetic 16 by 16 peptide array corresponds to the same number of micromirrors. The large size of peptide pattern and the separation facilitate biochip experiments using fluorescence assay. The peptide pattern has been synthesized on the GPTS-PEG200 surface with BSA-blocking and thereupon the background was acetylated to reject non-specific bindings. Hence, an averaged slope at the pattern edge has been distinguishably improved in comparison to patterning results from an aluminum micromirror.

**Keywords:** Aluminum Micromirror, Averaged Slope, GPTS-PEG200, Peptide Synthesis, Single Crystalline Silicon Micromirror Array

## 1. Introduction

Over the past several years there has been increasing interest in maskless lithography and its application using a micromirror array, such as DNA (deoxyribonucleic acid) array synthesis, peptide array synthesis and deep ultraviolet exposure[1-4]. In biochip fabrication systems, micromirrors have been used for the generation of various light patterns that correspond to each protein site. In particular, the use of a micromirror for a biochip fabrication is a great help to reduce numerous photomasks and improve the flexibility of pattern generations. In case of a micromirror in the TI DLP™ [5], its design target was focused on the projection display so as to have a small size and a high fill factor to implement high resolution. The TI DLP™ system is therefore, not the best choice for peptide synthesis application due to blurred UV light patterning [1]. Therefore, more than one row or column of mirror should be used as separation between biopatterns. In addition, 4 or 9 mirrors were composed to one biopattern since the mirrors are too small to detect a biopattern optically on their own. In the references [3, 4], micro mirror array was designed for biochip fabrication so that the micro mirror is 54 μm and the separation between each mirror is 30 μm. Therefore, one mirror could correspond to one peptide pattern. However, the aluminum mirror is still not very flat and the biopatterns are blurred. The single crystalline micro mirror array was proposed and fabricated for biochip fabrication [6]. A large mirror size (210 μm) and separation (80 μm) are suitable for biochip fabrication and the flatness is superior to the aluminum mirror.

We present herein a detailed result of peptide array synthesis using a single crystalline micromirror array and compare the results with the biopatterns fabricated using an aluminum micromirror array.

† Corresponding Author: School of Electrical Engineering and Computer Science, Seoul National University, Korea (yongkkim@snu.ac.kr)
* Image Development Team, Samsung Electronic Co., Ltd., Korea.
** Korea Electronics Technology Institute, Korea.
*** Department of Physics, Ewha Womans University, Korea.
**** School of Chemical and Biological Engineering, Seoul National University, Korea.
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## 2. Micromirror Design

Peptide synthesis application requires a uniform and simple micromirror for maskless photolithography. We sought to design and fabricate an adequately simplified but suitable micromirror as well as decrease the fabrication cost meeting requirements of the device performance and efforts. Hence, an appropriate structural material is important for a reliable device fabrication. Single crystalline silicon is selected as an applicable material for a micromirror from its properties such as negligible residual stress, high yield strength, high temperature resistance, and

flat surface in comparison to various metals. Its material properties and limitations have been taken into consideration during the design and fabrication procedure [6].

An electrostatically actuated micromirror consists of a mirror plate, torsional springs, and bottom electrodes as shown in Fig. 1. Reflective material, aluminum, is on top of a micromirror. The designed micromirror dimension is 210 x 210 $\mu m^2$. A spring is designed to be 1.2(W) x 6(H) x 42(L) $\mu m^3$. Resonance frequency and pull-in voltage are determined to be 10.92 kHz and 102.6 V, respectively.

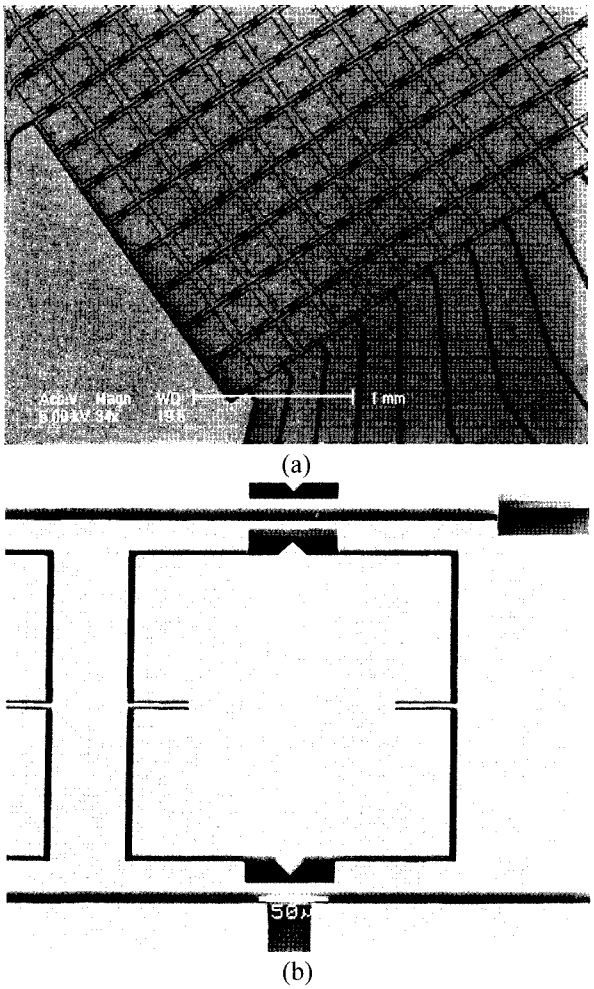


**Fig. 1.** SEM image of fabrication results. (a) A prospective view of a micromirror array. (b) A magnified view of a single micromirror

## 3. Maskless Photolithography System

Previously developed systems equipped with a micromirror array have special features for the biochip fabrication including protein or peptide synthesis to increase throughput and to reduce the labor and cost [3]. The maskless photolithography system consists of fine projection optics as shown in Fig. 2 in order to transfer desired patterns from a micromirror array to the biochip, fluidic components for chemical reaction and washing, and computer based control units for a user friendly system. After the desired bio-patterns and synthesis conditions are preset to the system, the system automatically performs the whole synthesis process on the glass chip. Although peptide synthesis has been performed previously using an aluminum micromirror array, we carry out peptide synthesis with a single crystalline silicon micromirror array in this work.

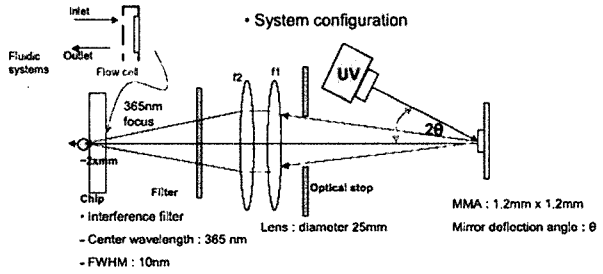


**Fig. 2.** Maskless photolithography system using a micromirror array [3]

Several process conditions were investigated for the optimized system. First of all, projection optics was modified to reject optical aberration and pattern deformation by an optical stop and proper lens combination. Not only the position of a micromirror array and UV light source were considered, but exposure conditions were also optimized. It takes an hour to complete covalent bonding reaction, thus the reaction chamber and injection tubes should be inert to used chemicals. At least, one side of the chamber should be transparent to remove photoliable functionals, for example photoliable nitroveratryloxycarbon

-yl (NVOC). Thus, the reaction chamber is fabricated using Teflon$^{TM}$ and has a sealed chamber by assembling a slide chip on the chamber. Since a small volume chamber is preferred to reduce used chemicals, the volume of the chamber is designed to have 130 $\mu\ell$. Nitrogen gas is used to pump up reaction chemicals and dry the slide chip after a single amino acid synthesis. The total operation is programmed on a graphical programming language called LABVIEW$^{TM}$ (National Instruments Co., Ltd.). The program not only controls mirror operations, it also manages chemical injection and drain, and stacking of each amino acid step by step. The graphical user interface allows the whole process control to be easy and effective.

## 4. Peptide Synthesis Process

Successive chemical reagents were used to treat the

glass surfaces in order to immobilize biomolecules. Glass slides were pre-cleaned in a mixture of $H_2SO_4$ and $H_2O_2$ (4:1) for 10 min and then rinsed with deionized water, ethanol, and dried in a vacuum oven. Silanizations with amino-functionalized silane (APTS) were carried out at 45°C in a solution of 5% (vol/vol) silane in chloroform for 2 hours. To remove the non-covalently adsorbed silane molecules, sonication in chloroform was performed for 10 min. The substrates were rinsed with ethanol and then blown dry with nitrogen. To introduce a spacer with a photolabile protecting group onto the aminated glass surface, we synthesized spacer molecules that have an amino group capped with a nitroveratryloxycarbonyl-polyethylene glycol (NVOC-PEG) protecting group. Thus, the aminated surface was exposed to a 5mM solution of NVOC spacer, benzotrialzol-1-yloxy-tris (dimethylamino) phosphornium hexafluorophosphate (BOP), 1-hydroxybenzotrialxole (HOBt), and diisopropylethylamine (DIEA) in dimethlyformamide (DMF) at 25°C for 2 hours. The samples were then rinsed with DMF, methylene chloride (MC), and dried by a nitrogen stream. After the coupling of NVOC-spacers to the glass surface, the UV light and the micromirror array were used to selectively remove the NVOC-protected group present on the glass surface in the reaction chamber. Then, biotin was coupled to the deprotected amine group on the surface by immersing the glass slide in a solution of 5 mM biotin, BOP, HOBt, and DIEA in DMF at 25°C for 2 hours. The glass slide was exposed to the phosphate buffer solution of FITC-conjugated streptavidin [7].

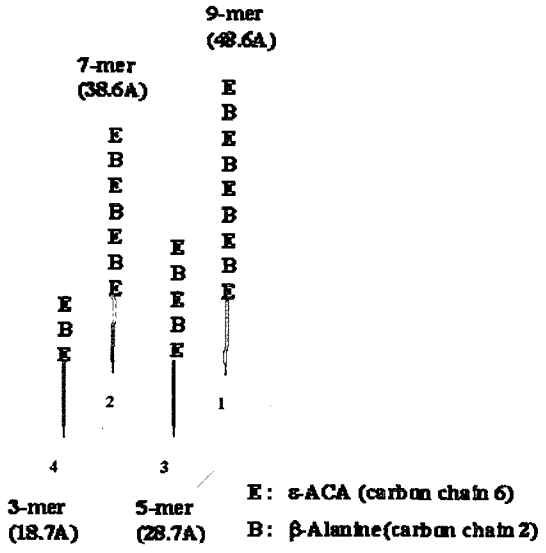


**Fig. 3.** Various spacers for spacer effects on protein interactions

In order to verify peptide interaction according to various spacer lengths, the described procedure is repeated until the desired spacer length is obtained. The spacers of four different lengths were synthesized using ε-aminocaproic acid (ACA) composed of six carbon chains and β-Alanine composed of two carbon chains. We denote ε-aminocaproic acid as 'E' and β-Alanine as 'B' in the diagram. Two kinds of spacers, E and B, were stacked alternatively as shown in Fig. 3. The first pattern has nine spacers, and the fourth pattern has three spacers (18.7 Å) composed of E-B-E.

## 5. Peptide Synthesis Results

Since we have ascertained the applicability of the peptide synthesis of a micromirror array in the reference [6], we wish to report the detailed results of the improvement of the maskless photolithography with a single crystalline silicon micromirror array.

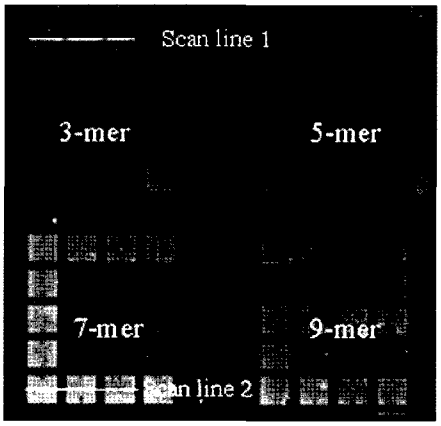


**Fig. 4.** Fluorescence image fabricated by the micromirror array

In Fig. 4, not only the fluorescent image of the synthesized peptide was observed, but also the large size of peptide pattern and the separation confirmed the expected result. Every pattern was distinguishable and easily detectable for fluorescence assay. The fluorescence intensity explains the strength of interactions between streptavidin and biotin, where each of the patterns have different spacer lengths. The longest spacer shows the brightest fluorescent intensity, and the intensity decreases as the spacer becomes short. From the fluorescence experiments, the relationships between the spacer length and the strength of interactions could be explained. The longer the spacer length is, the more the strength between streptavidin and biotin can be accomplished, since the FITC-conjugated streptavidin is such a large molecule that it is hard to bind to the biotin of which spacer length is short. The first four alphabets of Korean were demonstrated using the spacer generation procedure as stated above with the different spacer lengths.

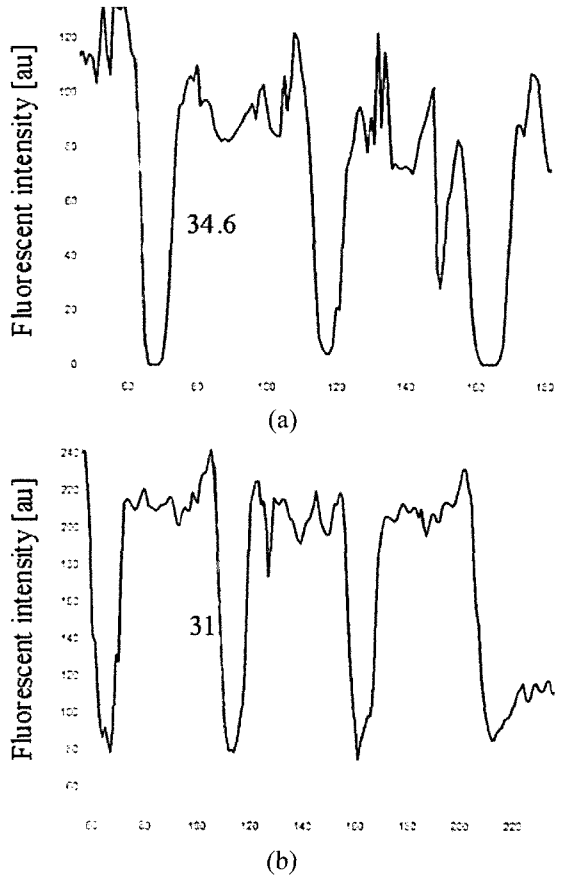


**Fig. 5.** Fluorescent intensity profile in Fig. 4 (a) along scan line 1, (b) along scan line 2

The peptide pattern and fluorescent intensity profiles using single crystalline silicon micromirrors are shown in Fig. 5. As described, a mirror size is 210 μm, and the separation between adjoining mirrors is 60 μm. In addition, the peptide pattern was made on the GPTS-PEG200 surface with BSA-blocking. The background was acetylated to reject non-specific bindings. The acetylated background is distinguishable from the process of the peptide synthesis using the aluminum micromirror array [4], which has suppressed noise signals at background successfully. In Fig. 5 (a), a background level is 0, and a peak level is 122 au. The separation level is 4 au, so the separation level is only 3.3% of the peak level with respect to the background level as a bottom level. The slope that represents sharpness at the pattern edge is calculated as about 34.6 au/μm. The same procedure could be applied to the second scan line as indicated in Fig. 5 (b), which shows similar results as Fig. 5 (a) and the slope is about 31 au/μm.

## 6. Discussion

Using the synthesized peptide patterns, the synthesis performance can be compared between previous aluminum micromirrors and the current single crystalline silicon micromirrors. The peptide pattern and fluorescent intensity profiles using the aluminum micromirror array are shown in Fig. 6. The mirror was totally fabricated with thermally evaporated aluminum. A mirror size was 54 μm, and the separation between adjoining mirrors was 30 μm. In addition, the peptide pattern was made using APTS-ACA spacers with BSA-blocking.

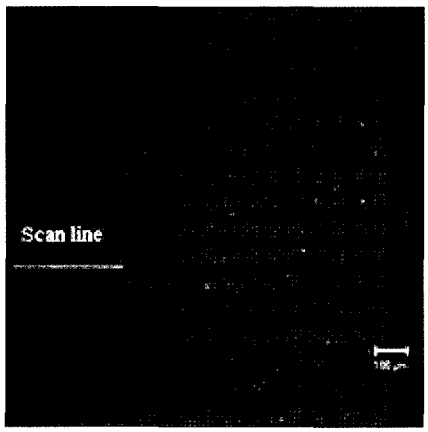


**Fig. 6.** Synthesized peptide patterns using an aluminum micromirror array

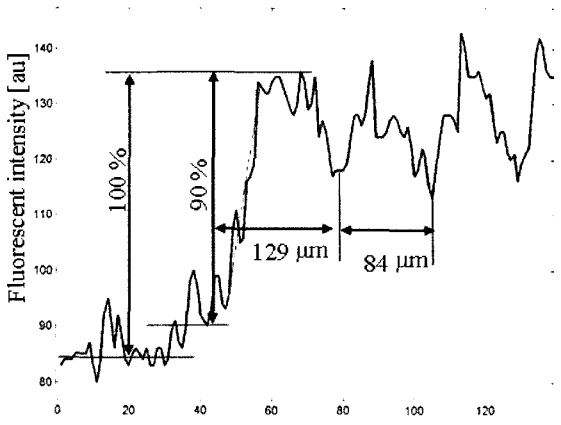


**Fig. 7.** Fluorescent intensity profile along the scan line in Fig. 6

In Fig. 7, a background level is 85 au, and a peak level is 136 au. The separation level is 113 au, so the separation level is 55% of the peak level with respect to the background level as a bottom level. Using this level information, the minimum separation can be found to be 81 μm to obtain the background level in the separation region. The slope at the pattern edge is calculated as about 2.2 au/μm.

From the experimental results, the pattern edge slope of results from single crystalline silicon micromirrors has been improved about 14 times more than aluminum

micromirrors, which can fabricate denser peptide patterns and improve the pattern recognition. The flat mirror plate that reduces scattered lights to the undesired sites, and the acetylated background that suppresses non-specific bonding, could be the reasons of edge slope improvements.

## 7. Conclusion

From the experimental results, a single crystalline silicon micromirror was found to achieve excellent characteristics for maskless photolithography. All designs of a devised micromirror were processed considering the material properties of single crystalline silicon. Mirror size was 210 μm and separation gap between micromirrors was 60 μm. We have performed peptide synthesis experiments so as to clarify the advance of the applicability of a single crystalline silicon micromirror and glass surface modification to maskless photolithography in comparison to an aluminum micromirror. The peptide pattern was made on the GPTS-PEG200 surface with BSA-blocking of which the background was acetylated to reject non-specific bindings. The spacers of four different lengths were synthesized. 34.6 au/μm slope at pattern edges using a single crystalline silicon micromirror were steeper than the other using an aluminum micromirror by about 14 times. Slope comparison result displays adequately separated peptide patterns with a single crystalline silicon micromirror array. A single crystalline silicon micromirror array turned out therefore to be a good research tool for maskless photolithography with glass surface modification in order to develop the bio chip fabrication.

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**Yun-Ho Jang** received the B.S., M.S. and Ph. D. degrees in the Department of Electrical Engineering and Computer Science from the Seoul National University, Seoul, Korea, in 1999, 2001, and 2005, respectively. His doctoral dissertation concerned robust the modeling, fabrication, and experiment of silicon micromirror array and its application in bio-molecular synthesis.

Since 2005, he joined the Samsung Electronics, Co., Ltd.., Yongin, Korea, as a senior researcher, where he currently participates in the development of CMOS image sensor, particularly in small pixel and high resolution active pixels.

**Kook-Nyung Lee** received the B.S., M.S. and Ph.D degrees in electrical engineering from the Seoul National University, Seoul, Korea, in 1998, 2000 and 2003, respectively. His doctoral dissertation concerned maskless photolithography system using micromirror array for protein chip fabrication. In 2003, he joined the Nano Bioelectronics Research Center, Seoul National University, Seoul, Korea, where he was a postdoctoral researcher involved with hand-held type fluorescence scanner for biochip. In 2005, he joined the Korea Electronics Technology Institute, where he is currently a senior research scientist. His current research

interests are nanowire and its application, especially nanowire based biosensor, nanowire FET, and nanowire TFT.

**Jae-Hyoung Park** received the B.S., M.S., and Ph.D. degrees in electrical engineering from the Seoul National University, Seoul, Korea, in 1997, 1999, and 2002, respectively. He was a Post-Doctoral researcher with the Inter-University Semiconductor Research Center (ISRC), Seoul National University from 2002 to 2004. He was also a member of research staff for the development of micromachined millimeter-wave device with the Center for 3-D Millimeter-Wave Integrated Systems, Seoul National University. He worked at Micro System group in the LG Electronic Institute of Technology as a chief research engineer from 2004 to 2006. In 2006, he joined the Ewha Womans University, where he is currently a full time instructor of the department of physics. His current research interests are focused on modeling, design, fabrication and testing of RF and optical MEMS devices.

**Dong-Sik Shin** received the B.S., M.S. and Ph. D. degrees in chemical engineering from the Seoul National University, Seoul, Korea, in 1999, 2001, and 2006, respectively. He is currently a Post-doc Researcher of School of Chemical and Biological Engineering in Seoul National University. His research interests are peptide synthesis, surface chemistry and biochips.

**Yoon-Sik Lee** received the B.S. degree in applied chemistry from the Seoul National University, Seoul, Korea, in 1974, and the Ph.D. degree in chemistry from Rutgers University, New Jersey, USA, in 1981. His doctoral dissertation concerned organic synthesis and surface chemistry. In 1981, he joined Chicago University, Illinois, USA, where he was a research associate with professor E. T. Kaiser involved with solid phase peptide synthesis. In 1982, he joined the Seoul National University, where he is currently a professor of School of Chemical and Biological Engineering. His current research interests are bioorganic synthesis, polymer chemistry, surface chemistry, nano-biotechnology and biochips.

**Yong-Kweon Kim** received the B.S. and M.S. degrees in electrical engineering from the Seoul National University, Seoul, Korea, in 1983 and 1985, respectively, and the Dr. Eng. Degree from the University of Tokyo, Tokyo, Japan, in 1990. His doctoral dissertation concerned modeling, design, fabrication, and testing of microlinear actuators in magnetic levitation using high critical temperature superconductors. In 1990, he joined the Central Research Laboratory, Hitachi Ltd., Tokyo, Japan, where he was a researcher involved with actuators of hard disk drives. In 1992, he joined the Seoul National University, where he is currently a professor of School of Electrical Engineering and Computer Science. His current research interests are modeling, design, and fabrication and testing of MEMS, and its applications, especially IMU (inertial measurement units), RF, optics and biotechnology.