ARBITRARY NANOPATTERNING INSIDE NANOCHANNELS BY INTEGRATION OF TOP-DOWN AND BOTTOM-UP APPROACHES FOR SINGLE MOLECULE ANALYSIS

Nobuhiro Matsumoto[1,2] and Yan Xu [1*]

[1] *Nanoscience and Nanotechnology Research Center, Osaka Prefecture University, JAPAN and*

[2] *Department of Physics and Electronics, Graduate School of Engineering, Osaka Prefecture University, JAPAN*

ABSTRACT

We developed method of arbitrary nanopatterning inside nanochannels by integration of top-down and bottom-up approaches which is technically supported by our glass room-temperature bonding and high precision position control techniques. With this method, we achieved protein nanopatterning in nanochannels, a core step of the nanochannel-based single molecule analysis.

KEYWORDS

Protein nanopatterning, Nanochannels, Glass, Self-assembly, Single molecule analysis

INTRODUCTION

A nanochannel provides appropriate space for single molecule analysis. In molecule analysis on planar devices, patterning is widely used to capture target molecules. Like conventional patterning in microchannel, patterning in nanochannels can be achieved using top-down approaches such as photolithography, after chip bonding which is usually performed at extremely high temperature (~1,000 °C) (Fig. 1a). However, due to the diffraction limit of light, it is practically difficult to make patterns smaller than 1 µm which are required to capture molecules at a single-molecule level. We recently developed glass low-temperature bonding and room-temperature bonding, making it possible to pre-integrate various functional materials which cannot tolerate high temperatures into nanofluidic chips [1, 2]. We also developed high-precision position control technique, which makes it possible to fabricate arbitrary structures inside a tiny nanochannel at a super high precision of 50 nm. Based on these two techniques, accordingly, we proposed a novel method of nanopattering in nanochannels by integration of top-down and bottom-up approaches, as shown in Fig. 1b. Briefly, the gold is pre-nanopatterned inside nanochannels by top-down approaches using electron beam lithography, deposition and lift-off that are guided by our high-precision position control technique. Then, the gold nanopatterned nanochannels are room-temperature bonded. After that, molecule nanopatterns are formed on pre-nanopatterned gold surface via molecule self-assembly, a classic bottom-up approach based on thiolation.

EXPERIMENTAL

Nanochannels were fabricated on glass substrate using electron beam lithography(EBL) and dry etching technique. The nanochnnels were 800 nm in width and 300 nm in depth. Next, various gold nanopatterns with arbitrary structures were fabricated in the nanochannels by EBL, deposition of Cr of 5 nm and Au of 100 nm and lift-off that were guided by our high-precision position control technique.

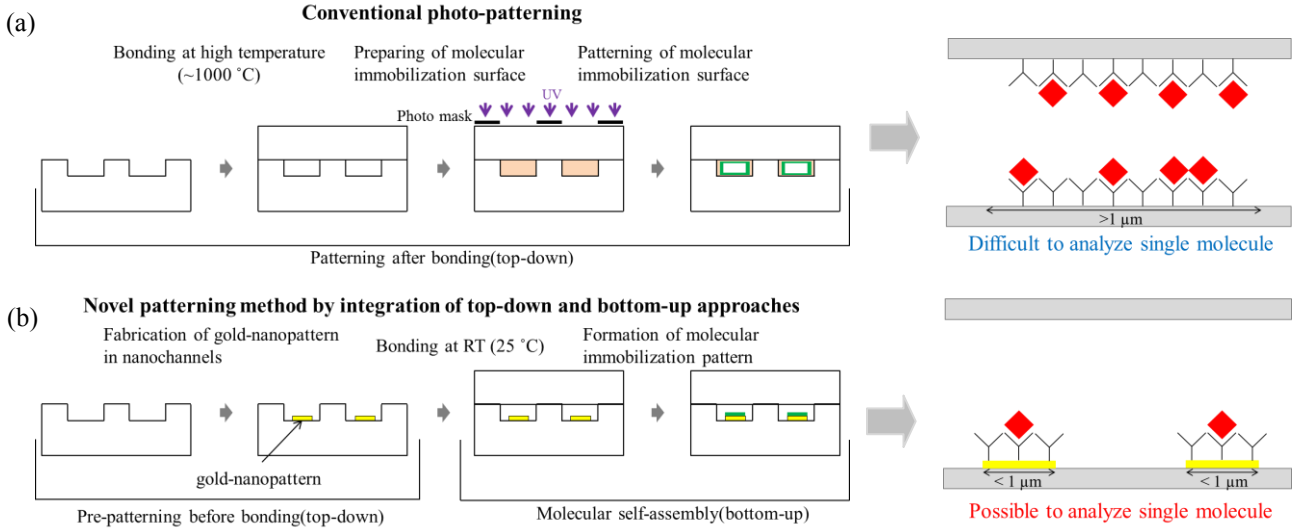

Figure 1: The concept of nanopatterning method in nanochannels by integration of top-down and bottom-up approaches for single molecule analysis

The gold nanoarray in a nanochannel was further used to pattern proteins in nanochannels, as a core step, i.e., protein immobilization at a nanoscales level, to realize the concept of nanochannel-based single molecule analysis proposed by us [3]. For easy detection, fluorescein isothiocyanate labelled bovine serum albumin (FITC-BSA) is used as a target protein. The FITC-BSA was immobilized on the gold nanopatterned surfaces via a self-assembled monolayer (SAM) having carboxyl head-groups (11-mercaptoundecanoic acid), which was performed on the gold, according to a protocol shown in Fig. 3a. The substrate was cleaned by immersion in piranha solution(7 : 3 volume ratio of concentrated sulfuric acid and H_2O_2) and were rinsed thoroughly with deionized water and 99.9% ethanol and dried with a stream of nitrogen. The substrates were immersed into 5 mM alkanethiol ethanolic solutions at room temperature for 24 h. After thorough rinsing in ethanol, substrates were treated with a solution of NHS (20 mM) and EDC (10 mM) in water for 30 min. The substrates were immersed in a solution of FITC-BSA (0.054 g/L) in PBS for 1 h at 37°C. Finally, protein (FITC-BSA) nanopatterning was confirmed by fluorescence microscope(BX53, Olympus).

RESULTS

Fabrication of zero-dimensional, one-dimensional, two-dimensional and three dimensional gold nanopatterns with arbitrary structures in nanochannels and multiple-patterns with different structures in a single nanochannel was achieved. Fig. 2 shows, for example, nanoarray (370 nm square) (Fig. 2a) and nanosegment (800 nm wide and 5 µm long) (Fig. 2b) in nanochannels of 800 nm wide and 300 nm deep. A gold nanodot array in a nanochannel (Fig. 3b) was further used to prepare protein nanopatterning according to the protocol shown in Fig. 3a. The strong fluorescence dots corresponding to immobilized FITC-BSA molecules were observed (Fig. 3c). In order to verify whether the patterning is powerful enough even at an extreme feature size, we specially designed the spaces between neighboring nanodots to be very small, smaller than that of width of nanochannel. The result (Fig. 3c) indicates that even in such a small feature size, the resolution of protein nanopattern was quite high. Therefore, we consider the method is very powerful for protein nanopatterning in nanochannels.

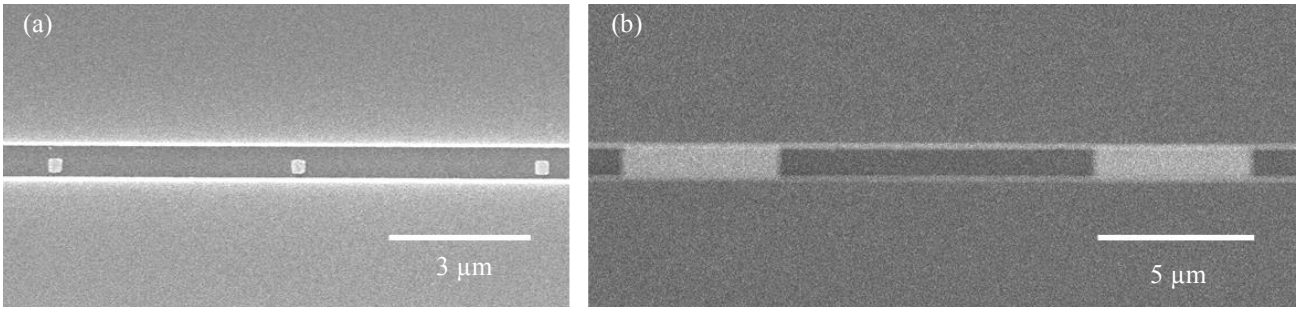

Figure 2: SEM images of gold nanopatterns in nanochannels. (a) nanoarray; (b) nanosegment

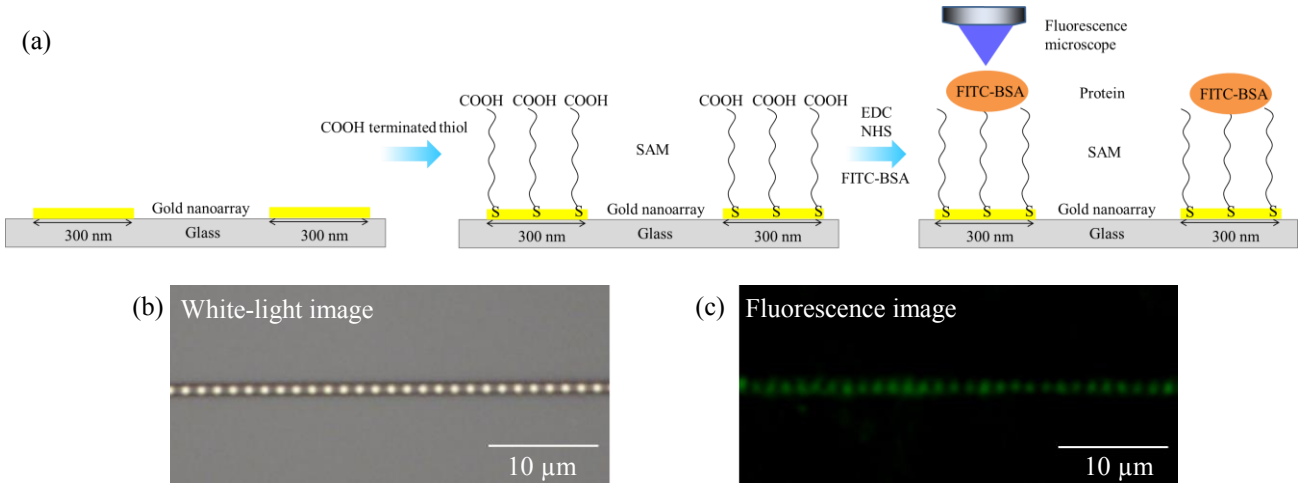

Figure 3: Nanopatterning of FITC-BSA in a nanochannel. (a)The protocol for immobilization of FITC-BSA on the gold surface of nanodot array in the nanochannel(b). (c)The fluorescence image of nanopatterned FITC-BSA in the nanochannel.

CONCLUSIONS

A method of arbitrary nanopatterning inside nanochannels by integration of top-down and bottom-up approaches was developed. The developed nanopatterning has superiority to conventional photo-patterning, such as, capable of fabrication of nanopatterns in nanochannels with arbitrary shapes, at any places with high precision, and of various molecules with high resolution.

ACKNOWLEDGEMENTS

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CONTACT

*Yan Xu, tel: +81-72-254-7813; y-xu@21c.osakafu-u.ac.jp