Extended Abstracts of the 2005 International Conference on Solid State Devices and Materials, Kobe, 2005, pp.458-459

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DNA Immobilization on Au/Sapphire Substrate Patterned by Nanolithography

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1. Introduction

We report a novel DNA sample preparation technique suited for scanning tunneling microscopy and spectroscopy (STM/STS). We fabricated a nanolithographic template consisting of nanoscale pores by electron beam lithography to prepare an ordered array of extended DNA molecules on Au surface, which is smooth at the atomic level. As prepared in this manner, each DNA molecule on the substrate is accessible without fluorescent labeling.

STM/STS is one of the potential analytical methods that enable us to clarify physicochemical properties of individual macromolecule from biological samples. Our final goal is the implementation of ultrafast DNA sequencing by STM/STS. There are some bottlenecks to be solved to implement this novel DNA sequencing method. One of the bottlenecks lies in sample preparation procedure. A sample mixture of DNA is normally in a liquid phase while STM/STS requires ultrahigh vacuum environment to achieve ultrahigh spatial resolution. A gyrated molecule of DNA must be extended into a straight chain so that we can analyze each base on DNA by STM/STS. To provide a solid-state sample of extended DNA molecules, molecular combing technique [1] and its relatives [2] have been developed so far. The arrangement of DNA molecules provided by those techniques is random and not predictable. Therefore it is essential to work on a large number of single molecules in order to reduce time required to find a single molecule on the substrate. Here we describe an improved technique for the sample preparation from a reduced sample, which enables us to reduce time-consuming jobs of finding a “needle in a haystack” since each extended DNA molecule is on a spot clearly defined by the nanolithographic process.

2. Fabrication

A sapphire wafer was used as a substrate to support DNA molecules. Au was thermally evaporated onto the wafer, and 150-nm-thick Au layer was planarized to the atomic level smoothness by wafer heating at 350 ˚C. Ra of the Au layer is 0.15 nm. 300-nm-thick electron beam resist (ZEP520A, Zeon Corporation, Japan) was spun on the Au layer. An ordered array of nanoscale pores with diameter ranging from 50 nm through 400 nm was created by electron beam lithography, and used as a nanolithographic template. Figure 1 shows the AFM image of 200-nm pores of the nanolithographic template on Au. Figure 2 shows the schematic image of our sample preparation procedure. λDNA fragments with length from 0.07 kbp through 19.33 kbp (Marker 6, Wako Pure Chemical Industries, Japan) was used as a DNA sample. 3’ terminus of λDNA fragment was derivatized with thiol group using 3’End Tag™ Kit (Vector Laboratories, Burlingame, CA).

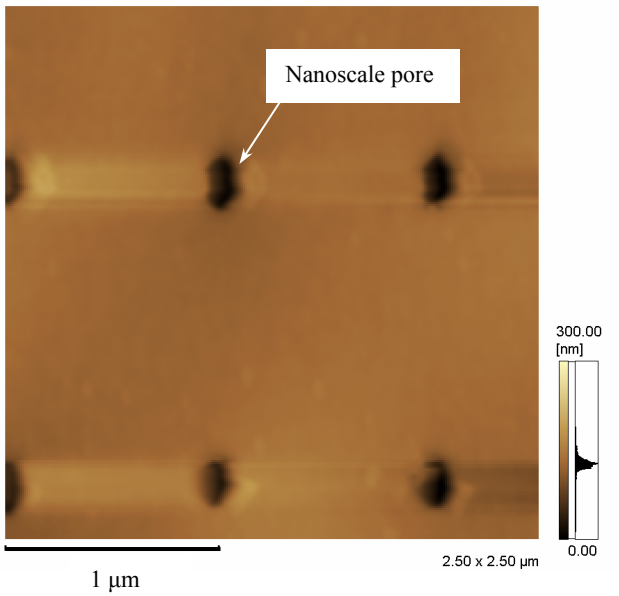

Figure 1 AFM image of the 300-nm thick nanolithographic template consisting of 200-nm pores. Au is exposed to the sample mixture at the nanoscale pores.

3. Experiment and result

A 20-µL droplet of thiol-derivatized DNA sample mixture was put on the nanolithographic template. The DNA molecules were concentrated on the nanoscale pores by electrophoretic migration at applied voltage of 5 V. The DNA was immobilized on Au by thiol-gold bonding (Figure 2 (i)). And then the nanolithographic template and excess DNA fragments were removed off from Au using N,N-dimethylamid. Figure 3 shows the AFM image of several double-stranded DNA molecules immobilized on Au. A drop of ammonium hydroxide was put on the substrate and boiled on 105 ˚C hotplate for 10 s to obtain single stranded DNA molecules following “DNA lift-off process”. And then the substrate was rinsed with DI water, and spin dry was done at 500 rpm for 30 s. During spinning, immobilized DNA molecules were stretched by centrifugal flow (Figure 2 (ii)), and finally settled down on the Au surface by capillary force (Figure 2 (iii)). As shown in Figure 4 (i), we have successfully extended a DNA molecule on clearly defined spot on the planar Au grain. The dimension of the extended DNA molecule is 0.5 µm in

length and 1.3 nm in height (Figure 4(ii)), which is almost equivalent to that of 0.93-kb single stranded DNA molecule.

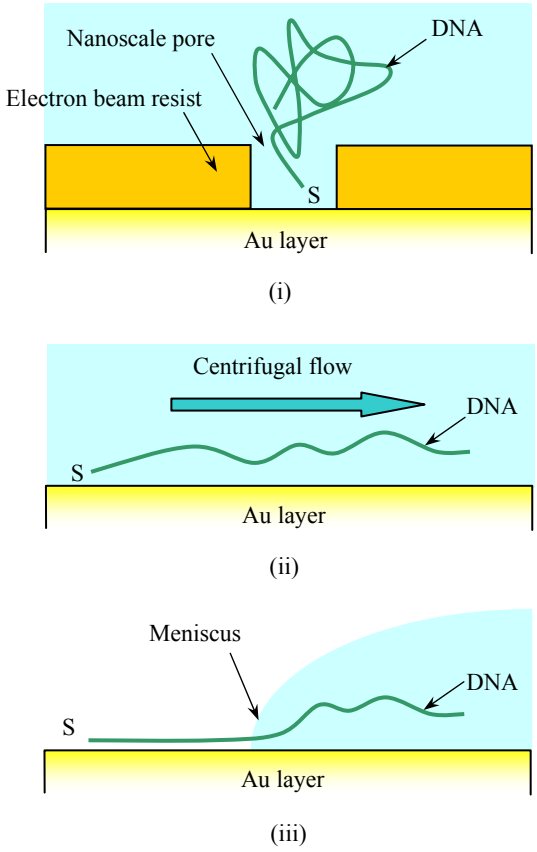

Figure 2 Schematic image of the sample preparation procedure; (i) Trapping at a specific site by DNA lift-off process, (ii) Extension by centrifugal flow, and (iii) Settling down by capillary force.

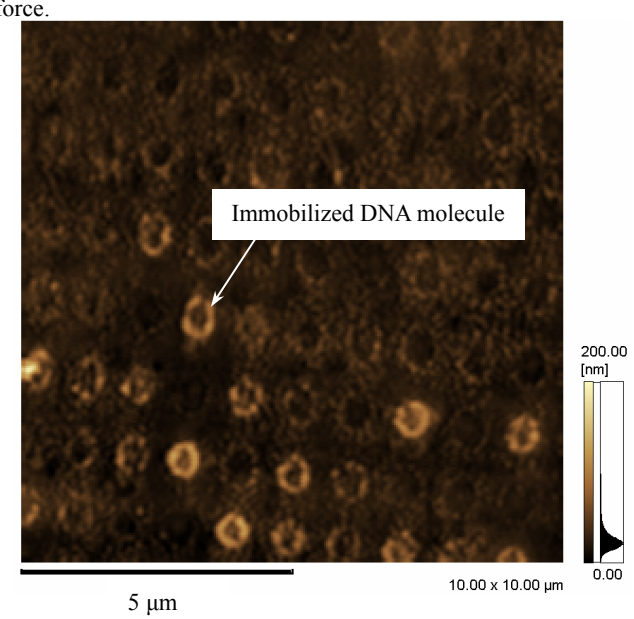

Figure 3 AFM image of dsDNA molecules immobilized at the sites of nanoscale pore.

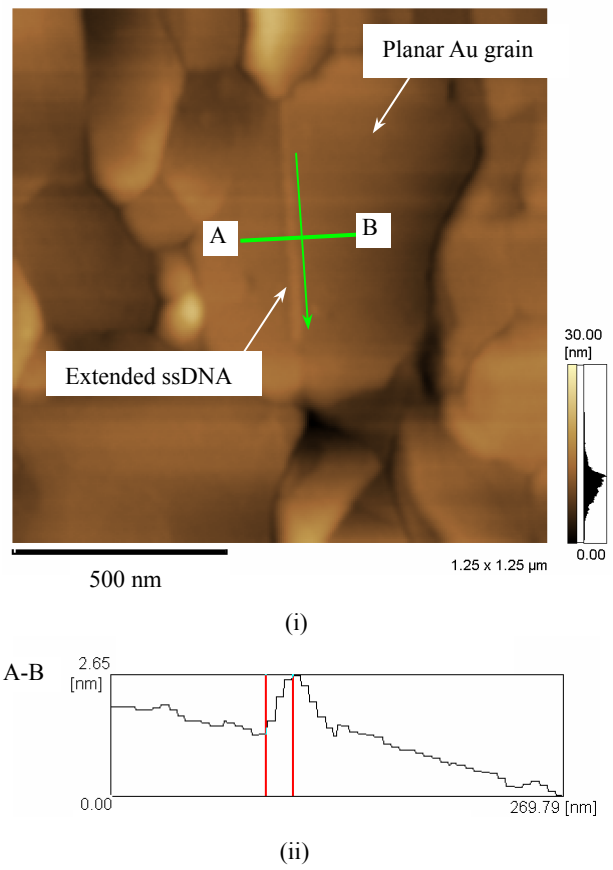

Figure 4 (i) AFM image of ssDNA extended on Au. (ii) Height profile of C-D cross-section.

4. Conclusion

We have fabricated an Au/sapphire substrate patterned with electron beam resist film. We successfully immobilized thiol-derivatized λDNA fragments through the 200 nm pores onto the patterned Au/sapphire substrate. We are characterizing the size dependence of immobilized DNA fragments on the pore size of the template

Acknowledgement

This work was financially supported by New Energy and Industrial Technology Development Organization (NEDO), Japan.

References

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[2] "Alignment and Sensitive Detection of DNA by a Moving Interface," A. Bensimon, A. Simon, A. Chiffaudel, V. Croquette, F. Heslot and D. Bensimon, Science, 265, pp 2096-2098 (1994).