Tunghai Science Vol. 19: 49-55
July, 2017

A Liquid-drop Method of Depositing DNA Molecules onto Annealed Gold Surface for Ambient Scanning Tunneling Microscopy

Jiun-Yih Ting*

Abstract

A liquid-drop method of depositing DNA molecules onto annealed gold surface for ambient Scanning Tunneling Microscopy is proposed in this report. Crowded DNA samples were observed. Different methods should be investigated for more adequate DNA distribution.

Keywords: Scanning Tunneling Microscopy, DNA, Sample Preparation, Annealed Gold

* Department of Applied Physics, Tunghai University, Taichung, Taiwan
E-mail address: sjyting@thu.edu.tw

1. Introduction

Using scanning tunneling microscopy in ultra high vacuum can image and distinguish individual bases in a single DNA molecule [1]. It is desired to do same kind of works in ambient condition [2]. However, not much progress was made due to lack of a routinely usable method of sample preparation for visualizing individual DNA molecules using scanning tunneling microscopy in air.

Substrate for DNA molecules contained solution should be inert to any chemical reaction, atomically flat in order to allow individual DNA molecules to be distinguished from background [3], and very good in conductivity for instrument to pick up rather weak tunneling current during scanning or even when covered by bio-molecules that are generally poor in conductivity. Moreover, individual DNA molecules should be distributed on substrate separately and immobilized for successful scanning.

A liquid-drop method of sample preparation was thus proposed to visualize individual DNA molecules on annealed gold surface using scanning tunneling microscopy in ambient condition.

2. Methods

2.1 Preparation of annealed gold strip

Gold foil of 0.1 mm thick was cut into 2 mm × 3 mm strips. They were cleansed by regular detergent solution with ultrasonic cleaner for 1 hr. Rinsed gold strips were placed inside a furnace at a temperature lower than but close to bulk melting point 1064°C for several hours. Annealed gold strip was glued with silver paint onto a mount for microscope. A mount with annealed gold strip was stored in a desiccator for later usage.

2.2 Preparation of DNA solution

Sample DNA, plasmid pUC19, was supplied at a concentration of 1,000 μg/ml in 10 mM Tris-HCl, 1 mM EDTA pH 8.0 at 25°C from New England BioLabs. DNA solution was diluted 500 times with distilled water twice. The working concentration of DNA is 4 ng/ml.

2.3 Deposition of DNA onto annealed gold surface

A drop of DNA solution was deposited onto an annealed gold strip. Then let gold strip be spinning at low speed, less than 500 rpm, for 10 min. The mount with already DNA treated annealed gold strip was stored in an oven at 50°C till later scanning. The schematic diagram of the scanning tunneling microscope is illustrated as in Figure 1.

3. Results

The generally used deposition method of DNA solution drop onto flat conducting surface can not be adapted for observing individual DNA molecules in ambient environment [4]. Figure 2 showed an image of very crowded distribution of plasmid pUC19. The sample was prepared with deposition of DNA solution drop onto pre-grounded annealed gold strip which was put on a spinner. Then spinner was rotating at 1,000 rpm for 30 min. The sample was stored at 50°C till later scanning. Although several pUC19 molecules showed up on the top layer of crowded background DNA aggregation as seen on the Figure 2, this result indicated that, by solution drop method, DNA molecules were pulled together due to the influence of surface tension between solution and air along the course of drying process. It was reported that the strength of force due to surface tension could even break double stranded DNA [5]. Alcohol can reduce surface tension of DNA solution [6]. Yet, DNA molecules tend to aggregate as solution drop dried gradually, even the surface tension was reduced significantly by alcohol.

When DNA solution was diluted with TE buffer, deposition method of a solution drop, dried in air, made another problem. Figure 3 showed some pUC19 DNA molecules were recognizable from crowded aggregation of pUC19 DNA molecules and TE buffer reagents. The background showed that terrace of annealed gold strip was fully covered by sediment of DNA and reagents mixture. Again, solution drop dried in air method could bring unwanted problems to the result.

4. Disccusion

The sample preparation method of depositing a DNA solution drop onto annealed gold surface is therefore not a proper way for visualizing individual DNA molecules using ambient scanning tunneling microscopy. Different methods should be investigated for further studies.

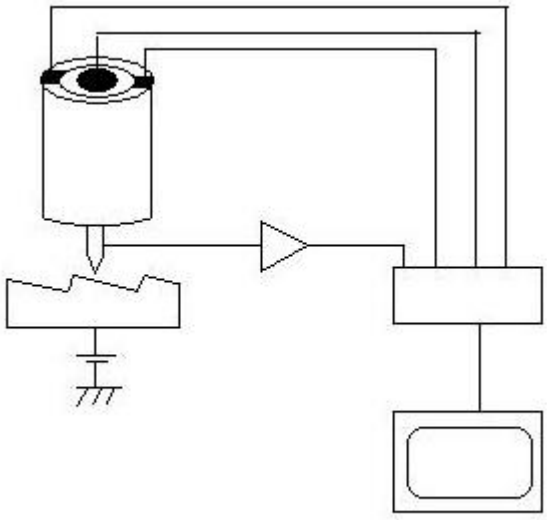

Fig. 1: The schematic diagram of the ambient scanning tunneling microscope.

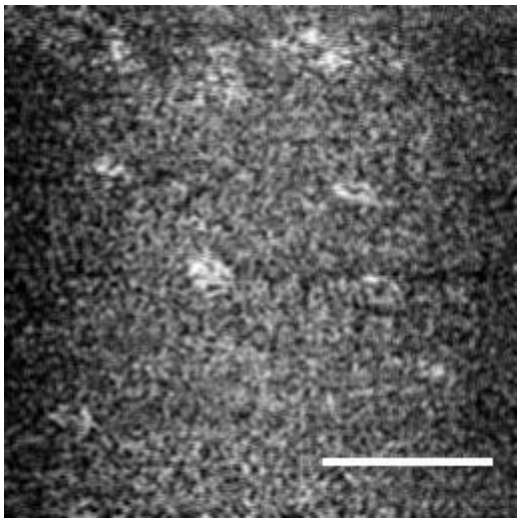

Fig. 2: The image was taken by STM in air, and its size is 1.5 μm × 1.5 μm. The scale bar represents 500 nm. DNA solution was diluted only with distilled water in this experiment. The sample was prepared by deposition method of a drop of DNA solution, dried in air. In this image, the plasmid pUC19 DNA molecules were pulled together by surface tension to form very thick and crowded layers.

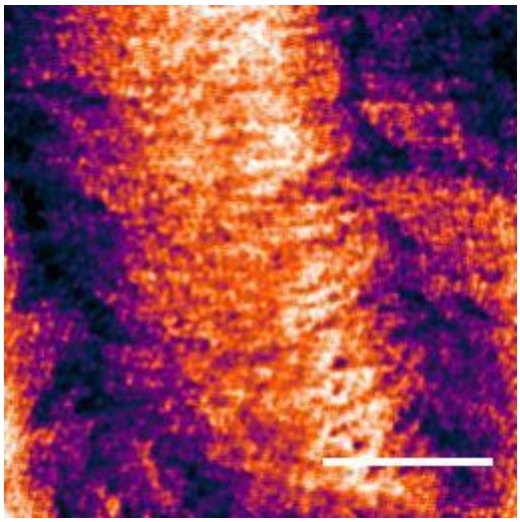

Fig. 3: The image was taken by STM in air, and its size is 1.5 μm × 1.5 μm. The scale bar represents 500 nm. Instead of using distilled water to dilute DNA solution, TE buffer was used to dilute pUC19 solution. The working concentration of pUC19 solution is 4 ng/ml. Deposition of DNA solution drop method was adapted to prepare pUC19 sample on annealed gold surface for scanning tunneling microscopy in air. In this image, some pUC19 DNA molecules were recognizable from crowded aggregation of pUC19 DNA molecules and TE buffer reagents. The background showed that terrace of annealed gold strip was fully covered by sediment of pUC19 DNA and reagents mixture.

5. References

[1] Tanaka, H., & Kawai, T. (2009). Partial sequencing of a single DNA molecule with a scanning tunnelling microscope. *Nature nanotechnology*, *4*(8), 518-522.

[2] Liu, L., Besenbacher, F., & Dong, M. (2014). Self-Assembly of DNA Bases via Hydrogen Bonding Studied by Scanning Tunneling Microscopy. In *Nucleic Acid Nanotechnology* (pp. 3-21). Springer Berlin Heidelberg.

[3] Clemmer, C. R., & Beebe, T. P. (1991). Graphite: a mimic for DNA and other biomolecules in scanning tunneling microscope studies. *Science*, *251*(4994), 640-642.

[4] Tanaka, H., & Kawai, T. (2003). Visualization of detailed structures within DNA. *Surface science*, *539*(1), L531-L536

[5] Bensimon, D., Simon, A. J., Croquette, V., & Bensimon, A. (1995). Stretching DNA with a receding meniscus: experiments and models. *Physical review letters*, *74*(23), 4754.

[6] Vazquez, G., Alvarez, E., & Navaza, J. M. (1995). Surface tension of alcohol + water from 20 to 50 degree C. *Journal of chemical and engineering data*, *40*(3), 611-614.

液滴法置放 DNA 分子於黃金表面供掃描穿隧顯微術觀測影像

丁君毅

摘 要

本報告探討液滴法置放 DNA 分子於退火黃金表面，以供掃描穿隧顯微術觀測影像。緊密糾結的 DNA 樣本被掃描成像。應檢驗不同的方法以得到最佳的 DNA 分布。

關鍵詞：掃描穿隧顯微術， DNA ，樣本製備，退火黃金