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Mist Method of Depositing DNA Molecules onto Annealed Gold Surface for Ambient Scanning Tunneling Microscopy

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Abstract

A method of depositing a stream of DNA-contained mist onto annealed gold surface is proposed to provide a simple and effective way for visualizing individual DNA molecules using ambient scanning tunneling microscopy (STM). Successful distribution of individual DNA molecules was achieved and imaged by STM in air. The result reported here implied that to investigate DNA molecules in detail in ambient environment would be practical.

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

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1. Introduction

The Scanning Tunneling Microscopy (STM) provided a special technique to investigate DNA molecules. Achievement of distinguishing individual bases in a single DNA molecule was accomplished by means of scanning tunneling microscopy in ultra-high vacuum [1]. In ambient environment, STM was not popularly used to investigate biological molecules. Obstacles lie in lacking of a routinely usable method to immobilize DNA molecules separately on good conducting substrate for scanning investigation [2].

The main problem that should be overcome is that surface tension between liquid solution containing DNA molecules and the air of the ambient environment [3]. Much effort was done to reduce the dragging force on DNA molecules through rinsing the sample with alcohol, or through drying sample inside low vacuum chamber [4]. However, the strength of force due to surface tension could even break double stranded DNA [5]. It is difficult to get rid of the surface tension under ambient condition.

An alternate method of depositing DNA molecules onto annealed gold strip was designed to avoid the aggregation of sample. DNA contained solution was converted to a stream of mist using doughnut shape ultrasonic humidifier [6]. Mist particles fallen on annealed gold strip were so tiny that they did not meet together to form a drop of liquid. Thus, DNA molecules resided within mist particles could be immobilized on gold surface separately. Since water molecules are volatile, individual DNA molecule would stay at the original location without aggregation.

A routinely applicable mist method of sample preparation was proposed to visualize individual DNA molecules on annealed gold surface using scanning tunneling microscopy in ambient environment.

2. Methods

2.1 Preparation of annealed gold strip

High-purity gold foil of 0.1 mm thick was cut into 2 mm $\times$ 3 mm strips. The top-right corner of gold strip was trimmed off a little bit to indicate the same correct side to be used in the experiment. They were cleansed by detergent solution with ultrasonic cleaner for 1 hr. Rinsed gold strips were placed inside a furnace at a temperature lower than bulk melting point 1064°C for several hours. This step was repeated for many times preceded experiment.

Annealed gold strip was glued with silver paint onto a mount for microscope and stored in a desiccator for later usage.

2.2 Preparation of DNA solution

Sample DNA, either plasmid pUC19 or bacteriophage ΦX174 DNA, was supplied by New England BioLabs at a concentration of 1,000 μg/ml in 10 mM Tris-HCl, 1 mM EDTA pH 8.0 at 25°C. DNA solution was diluted with distilled water down to working concentration as 4 ng/ml.

2.3 Deposition of DNA onto annealed gold surface

A drop of DNA solution was deposited onto an ultrasonic humidifier as shown in Figure 1. Solution drop became a stream of mist for 3 to 7 seconds usually. Let annealed gold strip on a mount be exposed to the mist at a distance of 15 cm away from the humidifier. Then let exposed gold strip be spinning at low speed, less than 500 rpm, for 10 min. The exposure process followed by spinning was repeated for several times. The mount with already DNA treated annealed gold strip was stored in a desiccator till later scanning.

3. Results

The DNA sample on annealed gold surface was imaged by STM in air. The scanning rate was 2 Hz. Figure 2 showed an image of individual DNA molecule of bacteriophage ΦX174 on terrace of annealed gold. The image showed here was background subtracted and color modified in order to enhance the clarity of the object.

Figure 3 showed an image of individual supercoiled DNA molecules of plasmid pUC19. Supercoiled DNA of pUC19 was observed as rod shape, not in circular form. This implied that ultrasonic humidifier might not make supercoiled DNA nicked or broken. Therefore, the sample preparation method described in this report can be useful for studies of intact state of DNA molecules or interactions between bio-molecules. The wavy curves seen as background in this image were again terraces of annealed gold in smaller scale. This was useful to identify DNA molecule and to confirm that there were no other contaminations in the sample.

4. Disccusion

The desired sample preparation method for STM in air to observe the individual DNA molecules should be simple and effective for routine investigation. Deposition of DNA

molecules onto annealed gold surface through a stream of mist was successful in observing individual DNA molecules by means of STM in ambient environment.

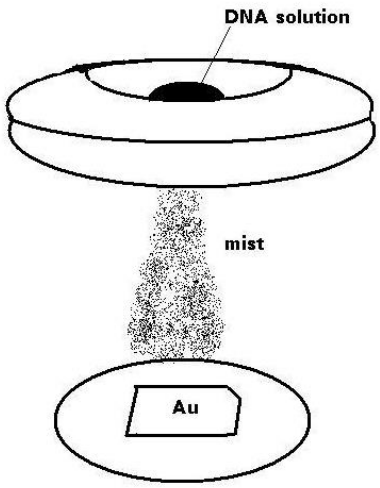

Fig. 1: The schematic diagram of a doughnut shape ultrasonic humidifier. A drop of DNA solution was deposited onto an ultrasonic humidifier. Solution drop became a stream of mist for 3 to 7 seconds usually. Annealed gold strip on a mount was exposed to the mist at a distance of 15 cm away from the humidifier.

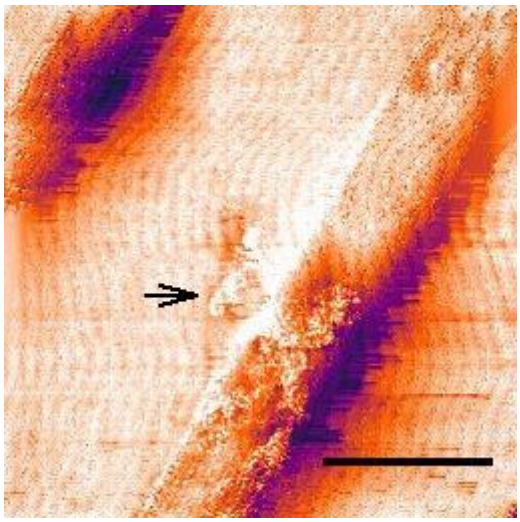

Fig. 2: The image was taken by STM in air and its size is 1.5 μm × 1.5 μm. The scale bar represents 500 nm. The annealed gold surface formed as terraces. An individual DNA molecule of bacteriophage ΦX174 was observed on gold surface near the edge of terrace as indicated by an arrow.

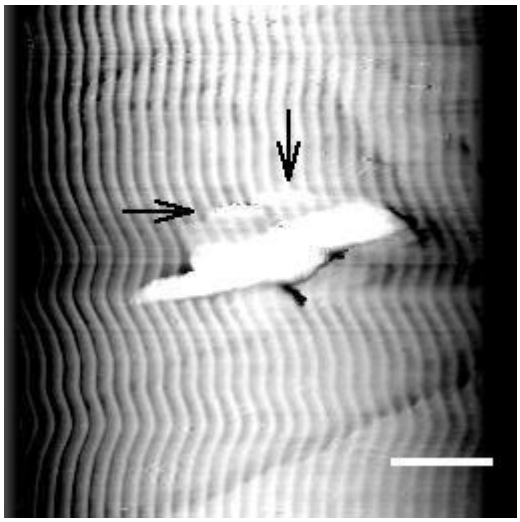

Fig. 3: The image was taken by STM in air, and its size is 0.5 μm × 0.5 μm. The scale bar represents 100 nm. Exposing annealed gold strip to a stream of DNA contained mist, two individual supercoiled plasmid pUC19 molecules (pointed by arrows) were close to aggregation of other pUC19 supercoiled DNA molecules.

5. References

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噴霧置放 DNA 分子於退火黃金表面供掃描穿隧顯微術觀測影像

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摘 要

本報告提出簡易有效的噴霧法，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。個別 DNA 分子已能成功散佈，並已掃描得到影像。實驗結果顯示於大氣環境中亦可細緻觀測 DNA 分子結構。

關鍵詞：掃描穿隧顯微術， DNA ，樣本製備，退火黃金