3D NANO WIRE-FRAME FOR HANDLING AND OBSERVING A SINGLE DNA FIBER

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ABSTRACT

We developed a new process, "3D-EBD (Electron Beam Deposition)" to form 3D wire-frame and nano-meter-sized structures. Using this process we formed a 20nm diameter nano-probe on the tip of a glass needle, and extracted a single DNA fiber from a human chromosome using it. We are also applying it to a single DNA observation using SNOM (Scanning Near-field Optical Microscope).

INTRODUCTION

Recently, DNA research has shown rapid progress causing high demand in new and efficient techniques for DNA analysis. Currently, skillful scientists analyze such genetic information as base sequence by chemically treating a large number of DNA in a solution filled with various enzyme[1]. Such lengthy indirect methods, however, require a series of processes and various reagents for treating DNA in groups.

Our direct method analyzes DNA efficiently by handling a single DNA fiber to observe a specific gene on it. Fig. 1 shows its concept. We pull one DNA fiber from a chromosome that decisively contains only a single DNA fiber. Using the probe, we extract and expand the complexly folded fiber from a chromosome. We then label a particular sequence or mutation on the fiber with fluorescent dye and observe it with SNOM.

This process requires the following two functions 1) to extract and expand a single DNA fiber removing all its entanglement, 2) to detect a fluorescent signal with high spatial resolution. Both of these functions require a probe end-effector with a nanometer-sized tip because a DNA fiber is 2nm in diameter and a nucleotide is 0.3nm long. Photolithography is a common technique for fabricating such nanometer-sized structures, however, even sacrifice-layer etching has difficulty in making 3D structures. We developed a new process, "3D-EBD (Electron Beam Deposition)" to directly make 3D and nanometer-sized structures for handling and observing a single DNA fiber.

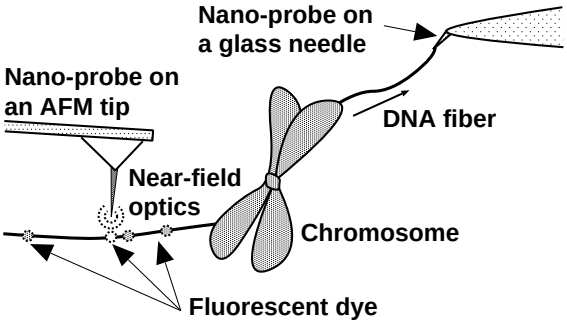

Fig. 1 Schematic of handling and observating a single DNA. A nano-probe on a glass needle extracts a single DNA fiber from a chromosome, and a nano-probe on an AFM tip detects a fluorescent labeled gene with near-field optics.

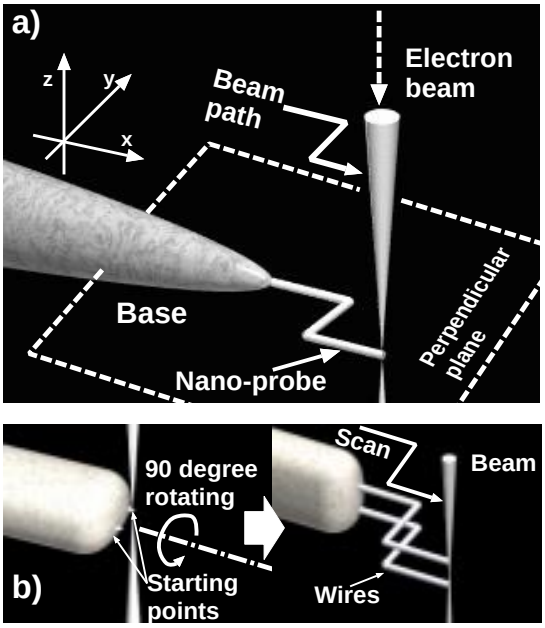

Fig. 2 Illustration of 3D-EBD process. a) A nano-probe is deposited in a plane perpendicular to the beam. b) Parallel wires are produced as follows; making starting points, rotating the base by 90°, and scanning the beam from the two starting points.

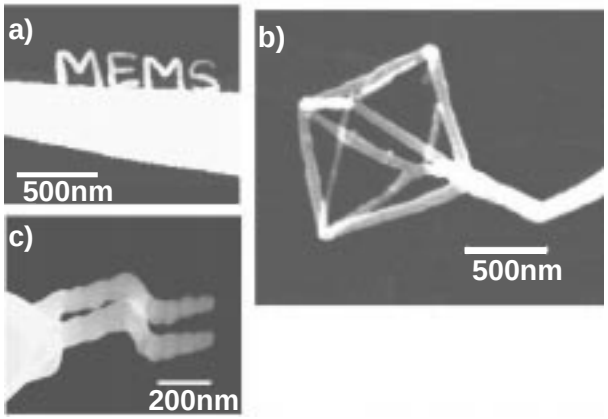

Fig. 3 Examples of 3D nano wire-frame made by 3D-EBD. a) Nano illuminations lettered *"MEMS"*. One character is about 250nm wide. Its wire has about 40nm diameter. b) A regular octahedron frame constructed with 100nm thick wire. Edge to edge length is 1μm long. c) Two parallel wires made by the process shown in Fig. 2b.

3D-EBD PROCESS

EBD is well-known as a sample contamination phenomenon during SEM observation; an electron beam excites and dissociates gas molecules, mainly consisting of carbon, hydrogen, and oxygen, in an SEM chamber; the dissociation deposits on object surfaces. In conventional methods, the molecule dissociation grows only in the direction opposite from the beam[2][3]. In contrast, with our 3D-EBD method, dissociation grows in a plane perpendicular to the beam (Fig. 2). A tens-nanometer thick carbon compound wire, "nano-probe" forms just along the beam path. By slowly controlling the beam to move from the edge of a base, we grow a wire along the beam path in space as designed (Fig. 3a). Additionally, by rotating the base, we can easily fabricate various 3D wire-frames (Fig. 3b). Moreover, starting from evenly spaced points, we can form a cyclical structure with one scan (Fig. 3c).

NANO-PROBE FABRICATION FOR SINGLE DNA HANDLING

For handling a single DNA fiber, we applied the 3D-EBD process to make a nano wire-frame on the tip of a glass needle covered with aluminum. We named the product "nano-probe".

Fig. 4 a) shows the hook-shaped nano-probe for catching and extracting a DNA from a chromosome or a nucleus. Its tip is about 20nm in diameter. The nano-probe shown in b) is a "C" shaped nano-probe designed for cutting and picking up a 300nm section from a DNA fiber.

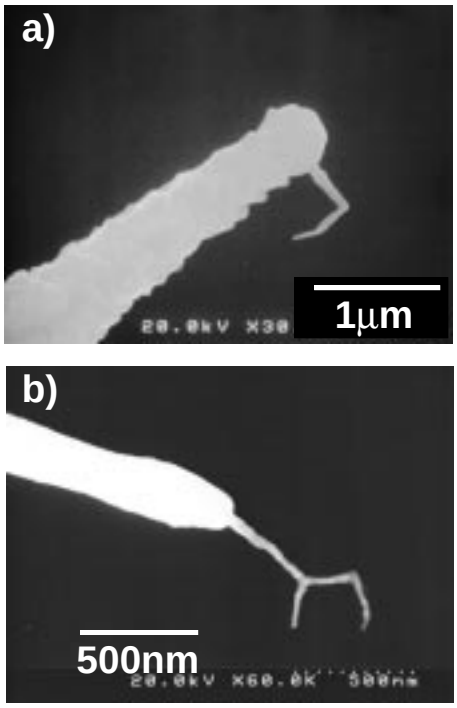

Fig. 4 SEM view of nano-probes. a) A hook-shaped probe. b) A letter "C" shaped probe.

A 3D micromanipulator (Narishige MHW-3) mounted on an inverted fluorescent allowed us to manually handled the nano-probe. We used YOPRO-1 iodide to dye DNA fiber samples.

DNA EXTRACTION USING NANO-PROBE

This section describes some of the experiments we ran. We first tried to extract a DNA fiber from a rice nucleus. We dropped phosphate buffer with rice nuclei onto a slide glass and dried it to fix the buffer. We then dropped detergent (0.5% Sodium Dodecyl Sulfate) solution to dissolve the nucleic membrane, hooked and extracted DNA fibers from a particular nucleus with the hook-shaped nano-probe. Fig. 5 shows this experiment. A single rice nucleus contains 24 DNA fibers so that the hooked DNA fiber can not be identified as a particular DNA fiber.

We repeated the same experiment using polyamine buffer with human chromosomes (K562). Fig. 6 shows the hook-shaped nano-probe unfolding the superhelical structure of DNA in a chromosome. In this experiment, we handled a single DNA fiber because each chromosome has only one fiber.

Next, we tried to pick up a specific fragment from a single DNA fiber. Fig. 7 shows the nano-probe cutting a single DNA fiber extracted from a nucleus with SAW (Surface

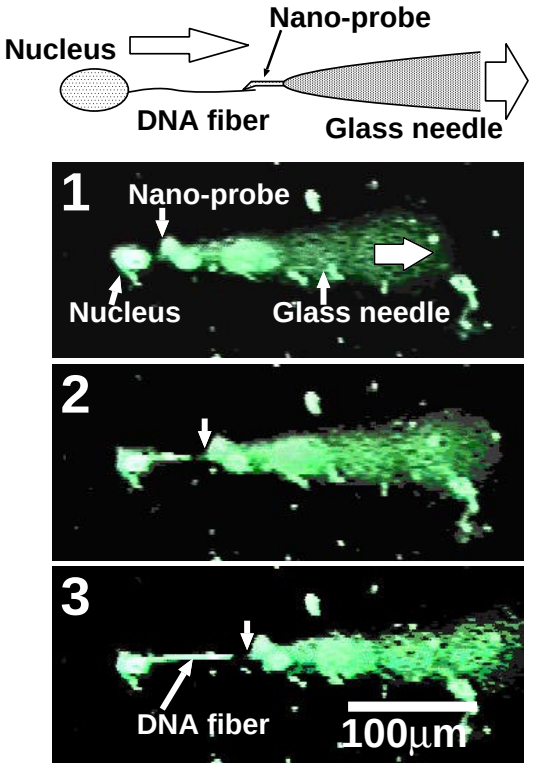

Fig. 5 Fluorescent microscopic view of extracting DNA fibers from a rice cell nucleus.

Acoustic Wave)[4] and also picking up the same fragment. We cut the DNA fiber mechanically by scraping it onto the slide glass using a straight nano-probe. Chemical interaction between aluminum and phosphoric acid radical of the DNA fiber picked up the fragment. In order to cut the fiber at a particular base sequence, we plan to coat the nano-probe tip with restriction enzyme. Moreover for reliable picking, we are planning to use chemical adhesive.

DNA OBSERVATION USING NP-SNOM

We are currently experimenting with nano-probe application to SNOM (Scanning Near-field Optical Microscope) in ordre to observe the DNA.

SNOM is a kind of SPM (Scanning Probe Microscope) with high resolution beyond conventional optical microscopes. SNOM with the nano-probe promises a much higher resolution. We call this "NP-SNOM (Nano-Probe-SNOM)".

Fig. 9 shows the nano-probe for the ultra high resolution SNOM. A nano-probe extends from the apex of the pyramid shaped silicon nitride cantilever for AFM (Atomic Force Microscope). By covering the nano-probe tip with metal, it will work as a point source of evanescent light.

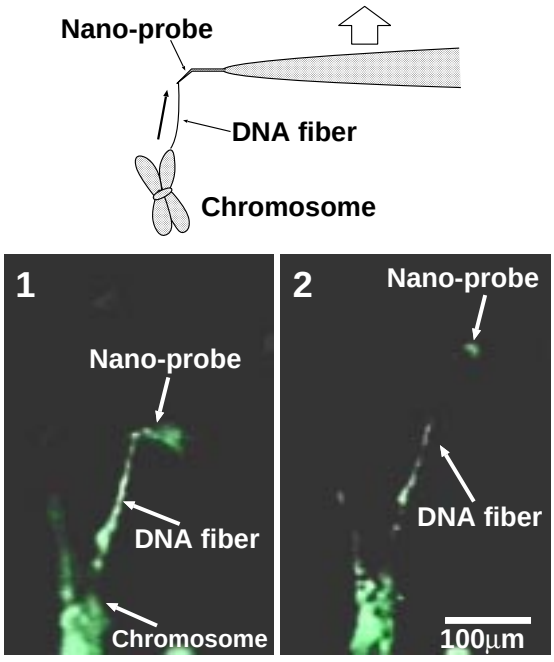

Fig. 6 Fluorescent microscopic view of extracting a single DNA fiber from a human chromosome.

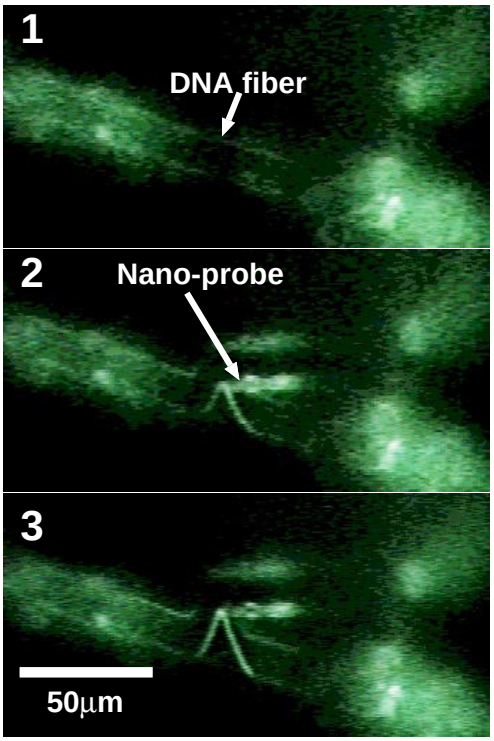

Fig. 7 Fluorescent microscopic view of picking up a particular DNA fragment from an extracted fiber.

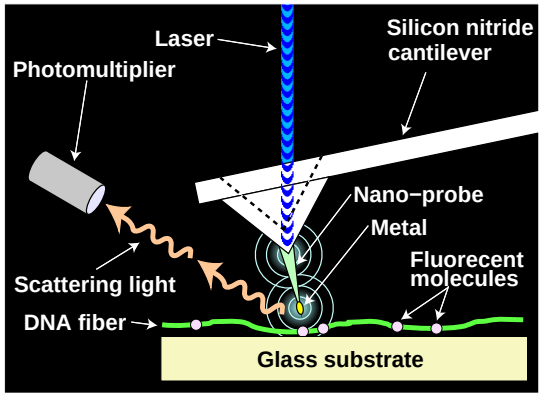

Fig. 8 Shematic of NP-SNOM

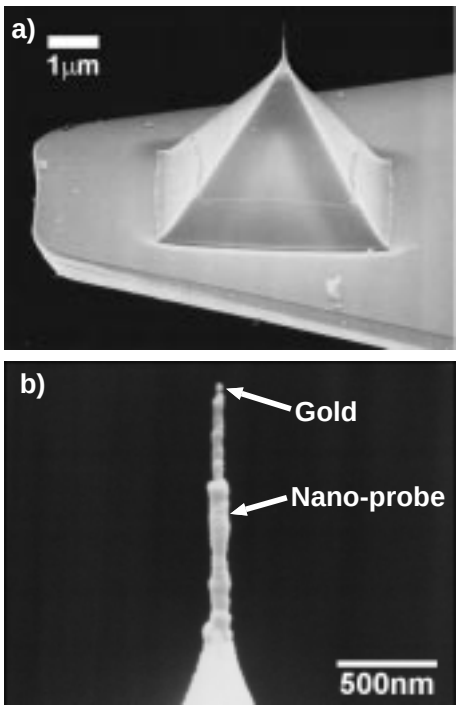

Fig. 9 a) SEM view of the nano-probe on the apex of the pyramidal silicon nitride cantilever. b) Its magnified view of the nano-probe vacuum evaporated by gold on the end.

The NP-SNOM light source is so small that we can illuminate a single molecule to distinguish it from others in observing fluorescent molecules on a DNA fiber. The expected high signal-noise ratio allows illuminating with a low power laser which prevents fluorescent molecules from fading.

We have successfully obtained SNOM images using the Si_3N_4 cantilever with the nano-probe (Fig. 10). Nonetheless, we have not yet captured a DNA fiber or fluorescent molecule image yet. We are continuing our efforts with a highly sensitive photo-multiplier and multi-wavelength laser.

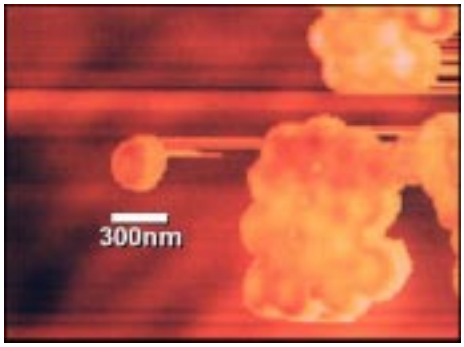

Fig. 10 SNOM image of 300nm diameter polystyrene particles using the cantilever with the nano-probe shown in Fig. 9.

CONCLUSION

We developed a new method ”3D-EBD (Electron Beam Deposition)” to make a 3D nano wire-frame called ”nano-probe” for handling and observing a single DNA fiber.

Experimenting with nano-probe demonstrated successfully DNA fiber extraction from nuclei / chromosomes and DNA fragments handling from long fibers.

A nano-probe on the tip of a silicon nitride cantilever for AFM (Atomic Force Microscope) obtained SNOM (Scanning Near-field Optical Microscope) images. We are continuing efforts to capture DNA fiber or fluorescent molecule images.

ACKNOWLEDGMENT

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