

DNA extraction from cell nuclei and DNA fishing using a 3D nano-probe

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1 Introduction

Now, rapid progress has been made in the research area of DNA, and new and efficient technique for DNA analysis is highly demanded.

In conventional methods of sequencing and recombination of DNA, many DNA fragments are cut from long DNA fibers on a nucleus with restriction nuclease; each fragments are sequenced by electrophoresis[1]. But with these methods, it is difficult to identify where a fragment was located in the long DNA fibers. We propose a new method to extract DNA fibers from a specific nucleus without cutting and to pick up a specific DNA fragment (we call it “DNA fishing”). This method simplifies the reconstruction process because the order of DNA fragments was known in fishing.

Fig. 1 illustrates the concept of our method. First, we extract DNA fibers from nuclei using a SAW(Surface Acoustic Wave) device. Next, we attempted DNA fishing with a nano probe made by a new EBD(Electron Beam Deposition) process.

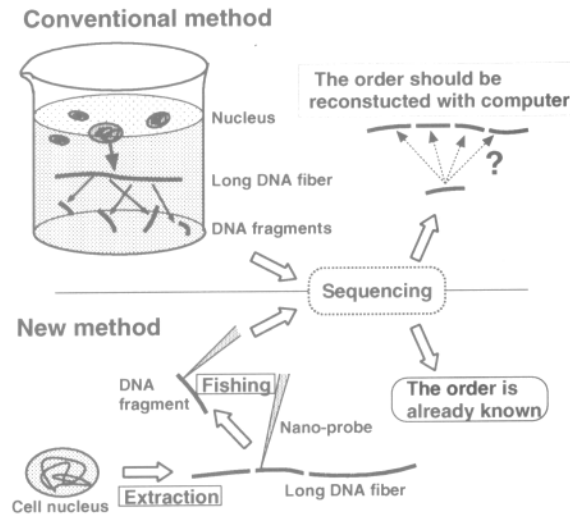


Fig. 1: Concept of the new method of sequencing DNA compared with conventional method.

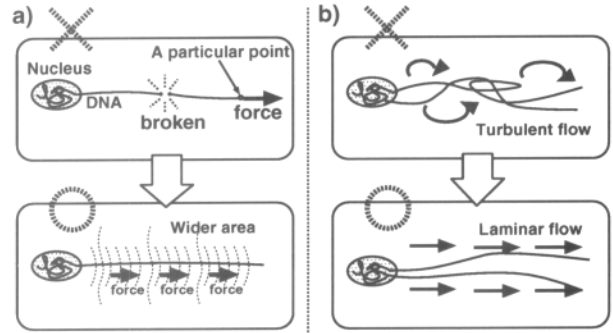


Fig. 2: Requirement for DNA extraction

2 DNA extraction

2.1 SAW device

The following two points are required for extraction and expansion of DNA fibers. 1) The force should affect not only at a particular point but also in wider area of the fiber, so as not to break the DNA fiber(Fig. 2a). It is suitable to apply a elastic-wave-like vibration to a long fiber. 2) The flow should be laminar so as not to entangle the fibers(Fig. 2b). The laminar flow can be generated by the method of dynamic molecular combing[2], but this method cannot identify the original nucleus where a DNA fiber was extracted. We employ SAW for DNA extraction.

SAW is high-frequency(10MHz-10GHz) vibration that travels only on the surface of a substrate. Vibration is generated by high-frequency electrical fields on a piezoelectric crystal.

We made an SAW device which has 16 pairs of $50\mu\text{m}$ wide electrodes on a LiNbO_3 substrate. The electrodes were fabricated by vapor deposition of Cr and Cu, and lift-off process (Fig. 3).

Fig. 4 demonstrates that the SAW device can generate a laminar flow.

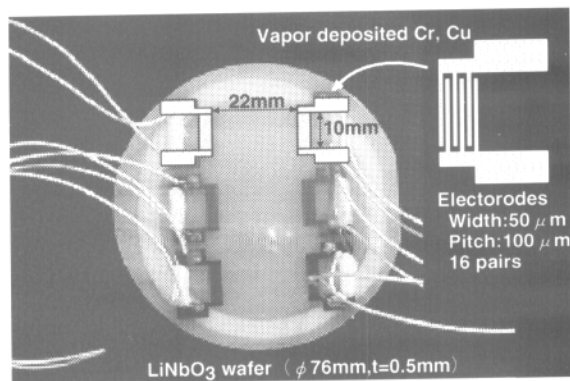


Fig. 3: SAW device; 6 electrodes made by vapor deposition of Cr, Cu on LiNbO₃

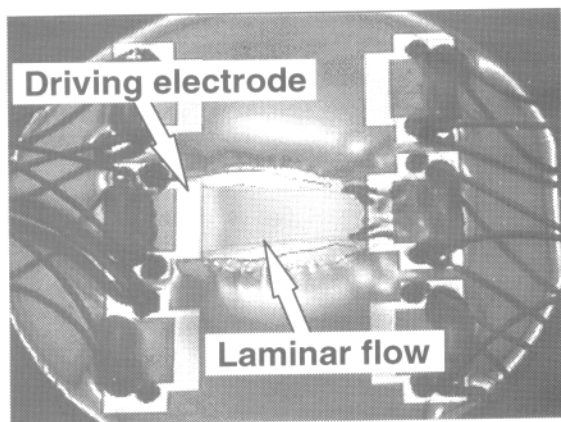


Fig. 4: Laminar flow generated by SAW device

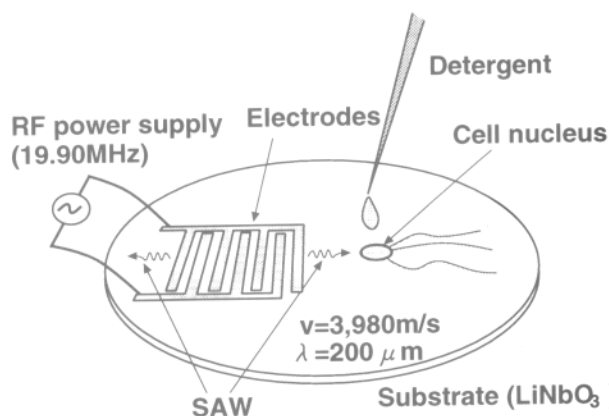


Fig. 5: Schematic of DNA extraction using SAW device

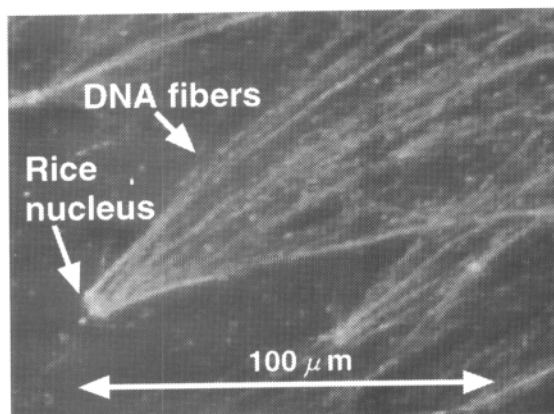


Fig. 6: Fluorescent microscopic view of the DNA fibers extracted and expanded from rice nuclei by SAW device. The fibers were not entangled.

2.2 Experiment of DNA extraction

We made the following experiment to extract DNA fibers from nuclei (Fig. 5). The phosphate buffer including rice nuclei was dropped onto the substrate and dried to be fixed. Detergent(0.5% Sodium dodecyl sulfate) was pipetted to dissolve the nucleic membrane. High frequency electrical field (20MHz, 1W) was impressed to the electrodes to generate SAW, and the solution flew along with the wave. In this way, DNA fibers were extracted and expanded about 0.5mm long (Fig. 6). The fibers spread fan-wise without any entanglement because the applied flow was laminar.

These DNA fibers should be about 10mm, so they are not perfectly expanded because they may still be wound onto histones. An enzyme to dissolve histones(Proteinase K) will be effective.

3 DNA fishing

3.1 Nano-probe for DNA fishing

A nano-probe for DNA fishing requires two essential functions. First, its tip should be nanometer-sized because a DNA fiber is 2nm in diameter and a nucleotide is 0.3nm long. Next, it should cut and pick up a DNA fiber. Optical tweezers is usually used for manipulation of a DNA fiber[3], but its spatial resolution is restricted by the diameter of optical beads(typical size is about 1μm). We developed a new process, "3D-EBD", to form the tip

of the nano-probe in a nanometer resolution, and attempted to coat the tip of the nano-probe with a restriction enzyme and a chemical adhesive to cut and pick up a DNA fiber.

3.2 3D-EBD

In EBD process, high energy electrons induce to dissociate molecules and the dissociation is adsorbed on a surface. The dissociation mechanism is complex and is not well understood because of the huge number of excitation channels available even for small molecules. However, EBD has been demonstrated as a new technique of lithography for many applications, a scanning tunneling microscope tip[4] and a field emission tip[5].

In conventional methods, the dissociation of molecules grows only in the reverse direction of the beam. But the new method can make the 3D feature as the dissociation grows on a perpendicular plane to the beam (Fig. 7). A tens nanometers thick wire of carbon compound is formed just along the beam path. When the beam is controlled slowly from the edge of a base toward a space, a wire grows along the beam path in the space as designed (Fig. 8). Additionally, rotating the base, we can easily fabricate various wires with 3D structure (Fig. 8(c,d,e)). The 3D-EBD examples were fabricated in vacuum of about 10^{-6} Pa using a FE-SEM of HITACHI S-4000 at 15kV.

3.3 Experiment of DNA fishing

We made a nano-probe of 20nm in diameter, 1μ long on the tip of a glass needle of 1μ in diameter and covered with aluminium (Fig. 9 a).

Then we coated the nano-probe with an amorphous teflon film, and strip it on the tip of the nano-probe with electron beam in an SEM. Avidin was fixed on the bald tip to fish a biotin labeled DNA fiber. In this case, avidin-biotin interaction was utilized because it is a very strong biological binding.

We made an experiment of DNA fishing; pipeted bacteriophage T4-DNA ($54\mu\text{m}$ long) solution ($0.2\text{ng}/\mu\text{l}$) on a slide glass; pipeted YOYO-1 iodide; set the probe as to put its tip inside the solution; covered with a cover glass; and observed with an inverted fluorescent microscope.

As a result, the tip of the probe hooked up a single DNA fiber flowing in the solution (Fig. 10). This figure shows that the probe can fish a single DNA fiber. We also plan to cut a long DNA fiber at

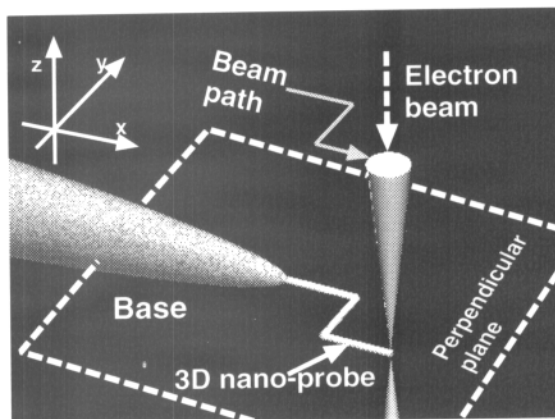


Fig. 7: Schematic of 3D-EBD method

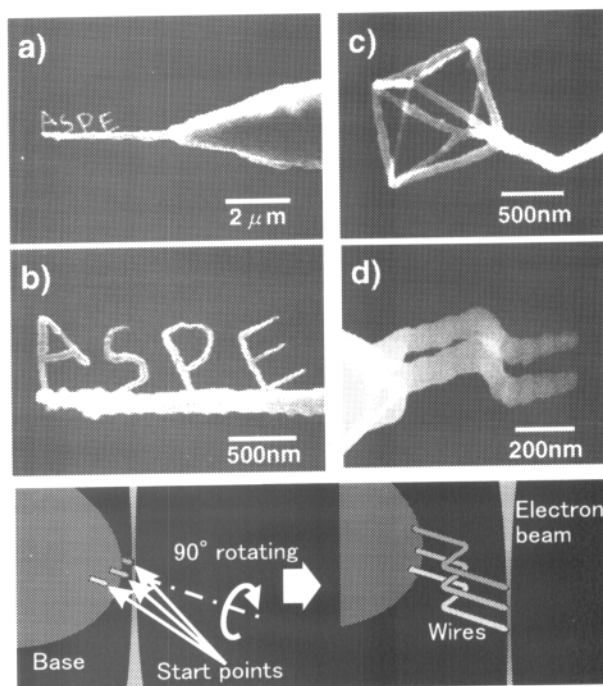


Fig. 8: Examples of 3D-EBD: a,b) Nano illuminations lettered "ASPE". Dimension of one character is about 400nm, and the line width is about 40nm. c) A regular octahedron frame, each side is $1\mu\text{m}$ in length, consisting of 100nm thick wire. d) Three parallel wires. e) The process of fabricating parallel wires; making start points, 90° rotating the base, making the beam scan from the start points.

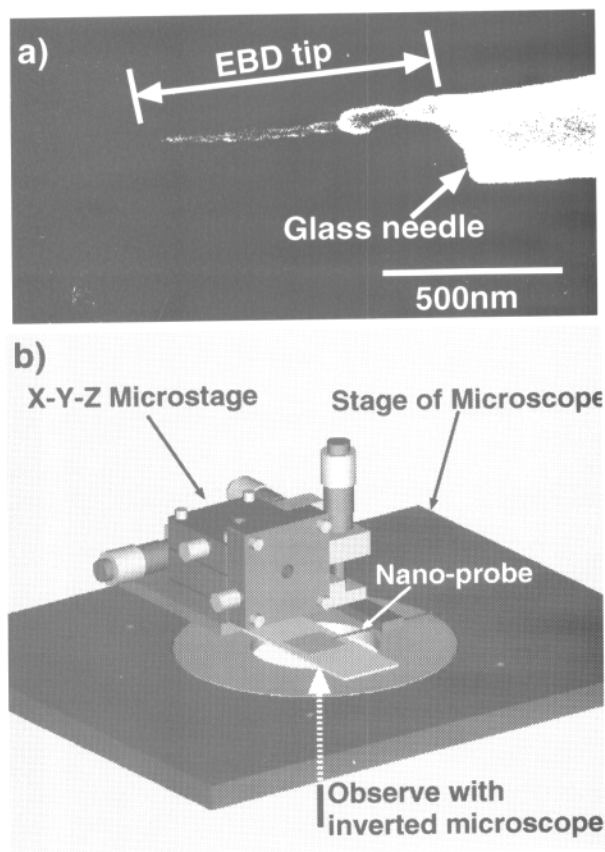


Fig. 9: a) SEM view of the tip of the nano-probe made by 3D-EBD method on the glass needle with hydrophobic coating . b) Schematic of DNA-fishing Experiment; we fixed the nano-probe on the X-Y-Z microstage moved its tip into the solution of the DNA fibers, pipetted on a slide-glass fixed on the stage of microscope, and observed from below with a inverted fluorescent microscope.

desired point using a probe coated with restriction enzyme.

4 Conclusion

To easily reconstruct the order of DNA fragments, a DNA extraction and DNA fishing is proposed.

DNA extraction was achieved on a LiNbO_3 substrate with a SAW(Surface Acoustic Wave) device, and DNA fibers of rice were expanded up to 0.5mm length.

Next, DNA fishing was achieved by a 3D nano-probe. We developed a 3D-EBD(Electron Beam

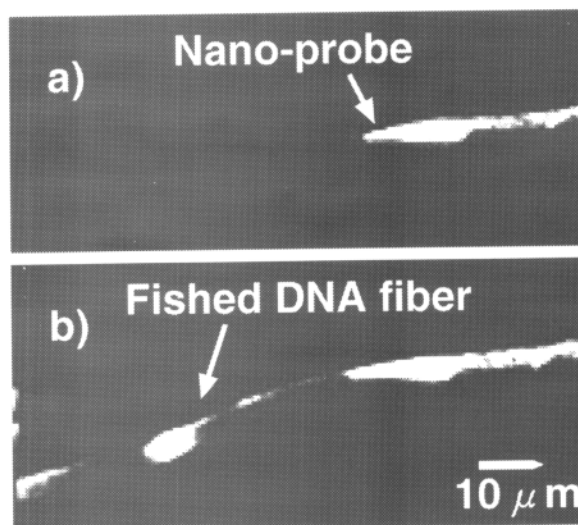


Fig. 10: Fluorescent microscopic view of the DNA fishing; a) Nano-probe before experiment b) Fished a T4 phage DNA fiber of $50\mu\text{m}$ long by the nano-probe

Deposition) process. We applied chemical modification to the tip of a nano-probe and fished a specific DNA fiber by the probe.

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