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Development of a new detection method for DNA molecules

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Abstract—A highly sensitive analysis method for biological molecules has been requested accompanied with recent development of molecular biology. Conventional highly sensitive detection methods are based on labeling techniques of fluorescent dye or enzyme. However, those techniques involve some problems on instability of signal. Because SQUID is an extremely high sensitive magnetic sensor, it can be applied to high sensitive detection of DNA labeled with magnetic small particle. The signal from SQUID is stable in contrast to fluorescent dye and enzyme, therefore it permits long time and high sensitive measurement. To demonstrate availability of this method, the sample coverslip on which the magnetic small particles were anchored through biotin labeled DNA was prepared. Scanning of high Tc SQUID sensor on the cover slip demonstrated that magnetic flux on the cover slip agreed well to the pattern of labeled DNA anchored on the coverslip. This result suggests that SQUID can be applied for specific detection of DNA molecules, especially for detection of DNA chips.

I. INTRODUCTION

Analysis of biological molecules is based on separation and detection. The detection method for the separated molecules determines the total sensitivity of analysis system. To improve the sensitivity, several methods have been introduced for the detection. Radioisotope labeling method is a one of most common methods [1], however it requires a special laboratory which confines radioisotope. Fluorescence labeling method is very high sensitive. It can detect only single molecules, however quenching of fluorescence dye inhibits long time observation [2]. Enzyme labeling method also achieves high sensitivity, however enzymes are sometime unstable [3].

Recently a labeling method with small magnetic particles has been introduced. SQUID has a large potential to detect small amount of these particles because of its high sensitivity for magnetic field. Detection of small magnetic particles with a SQUID for immunoassay applications is performed in several groups [4]-[5]. Even if particles are made of iron oxide, if their size becomes smaller, they show superparamagnetic properties. Therefore, some magnetic field should be applied to the particles for detection because they have almost no permanent magnetic dipole at room temperature. Koetits et.al. applied a pulse field to the particles and then measured the field decay from the particles in the range of msec. Empuku et.al. measured the field from the particles under a DC magnetic field. We measured the field from the particles under an AC magnetic field.

Recently DNA chip technique has been developed to analyze polymorphism of human genome. To apply high Tc SQUID sensor for DNA chip detection, we have developed a detection system and a sample preparation method.

II. EXPERIMENTAL METHODS

A. Detection System

The schematic diagram of the system is shown in Fig. 1. The SQUID is made of $Y_1Ba_2Cu_3O_{7-y}$ thin film and fabricated at Sumitomo Electric Industry and modified at our university [6]. The junctions utilized in the SQUID are of the step-edge type. The washer size of the SQUID is about 2.5 x 1.0 mm^2 and the effective area is 0.068mm^2. The SQUID was operated in a flux-locked loop with a flux modulation frequency of 100kHz. The magnetic flux noise in the white noise region was about 40 $\mu\phi_0/Hz^{1/2}$. The cryostat was specially designed for a SQUID microscope. The SQUID was located inside a vacuum and separated by a quartz window. A more detailed description can be found elsewhere [7]. Two coils (Helmholtz type) were mounted just above the SQUID microscope. A coverslip sample was conveyed into the coils by a motor driven string. A sinusoidal AC current with a frequency of 100Hz was directed to the coils; the magnetic field generated from the coil was modulated by the frequency. The modulated signal associated with the particle motion was then demodulated by the lock-in-amplifier. The lock-in-amplifier is home-made and consists of a phase sensitive detector, a phase shifter and a low-pass filter [8]. The rolloff frequency of the filter, which sets the bandwidth was 3 Hz (time constant

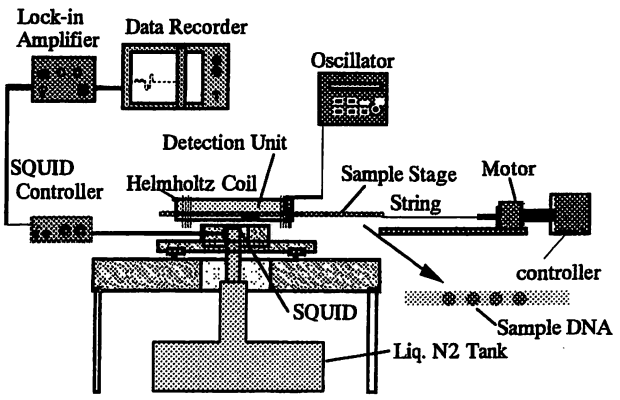

Fig.1 Schematic drawing of high-Tc SQUID detection system.
A magnetic field generated from the coils was modulated by 100Hz signal. A motor driven string was employed to convey the coverslip into the coils.

τ=0.33 sec). Since the rolloff gives you sensitivity to noise only within 3Hz of the desired signal, the signal/noise ratio is improved. The phase shifter was adjusted to give the maximum output signal. The use of the lock-in-amplifier is a crucial point to obtain a good resolution in the system. In this scheme, as with signal averaging, the effect of the modulation is to center the signal at the modulation frequency 100Hz, rather than at DC, in order to get away from 1/f noise, which occurs usually in the range from DC to 1 Hz. The two identical 1000 turn wound coils were spread apart with a distance of 50mm. The SQUID position was carefully adjusted before measurement so that the SQUID output signal without particles became zero. After the adjustment, the system was ready to measure the magnetic field from the particles.

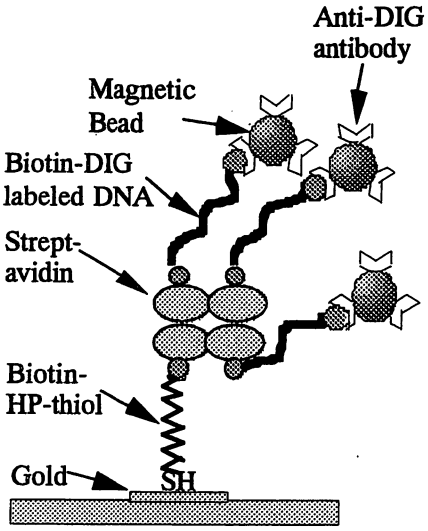

Fig.2 Schematic Drawing of anchoring of DNA

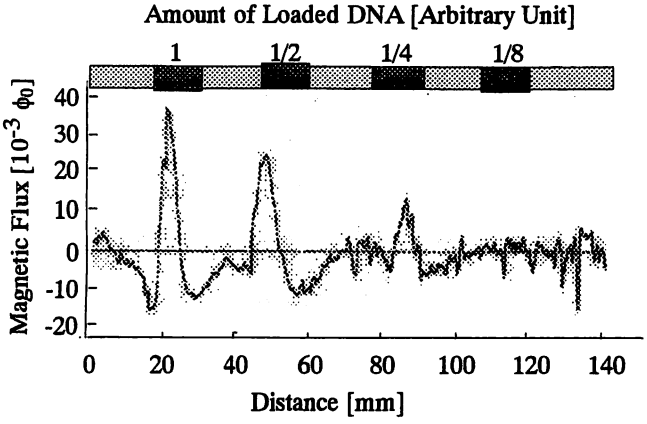

Fig3. Scanning of a DNA loaded coverslip.

B. Preparation of coverslips with DNA

Both terminals of DNA were modified with different labels, biotin and digoxigenin (DIG). This labeling was carried out using PCR (Polymerase Chain Reaction) with two different primers labeled with biotin and DIG.

The labeled DNA was anchored on gold layers patterned on a glass coverslip as shown in Fig.2. First of all, biotin was fixed on a gold surface through thiol-group, then avidin was fixed through avidin-biotin complex. Because avidin has four sites for biotin binding, the labeled DNA was anchored through the remained biotin binding sites of the avidin. The other label, DIG, was used for labeling with small magnetic particles. DIG was bound to the magnetic particles through anti-DIG antibody. The prepared coverslip was scanned by the high Tc SQUID detection system.

III. Results and Discussions

A series of the amount of DNA was prepared on a glass coverslip. The series consisted of 1, 1/2, 1/4. 1/8 of DNA in an arbitrary unit. The coverslip was scanned by the high Tc SQUID system. Figure 3 shows the result of scanning of the coverslip. The pattern of magnetic flux detected by high Tc SQUID agreed to that of the loaded DNA.

A system for detection of biological molecules using high Tc SQUID magnetmeter is proposed. The system is based on a magnetic modulation method, which had been developed for detecting a cluster of ultra small iron particles in a human body. The system succeeded to detect DNA molecules arranged on a glass coverslip. Because this is a prototype of DNA chip, this result suggests this system can be applied to detection of DNA chip.

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