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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 is required because of recent developments in molecular biology. Conventional highly sensitive detection methods are based on labelling techniques using fluorescent dyes or enzymes. However, since those techniques involved some problems with signal instability, a new technology has been sought. Because the superconducting interference device (SQUID) is an extremely highly sensitive magnetic sensor, it can be applied to the highly sensitive detection of DNA labelled with small magnetic particles. The signal from SQUID is stable in contrast to fluorescent dyes and enzymes, therefore it permits highly sensitive measurement over long periods of time.

Sample coverslips on which the small magnetic particles were anchored using biotin labelled DNA were prepared to demonstrate the availability of this method. Scanning the high-T_c SQUID sensor on the coverslip demonstrated that the magnetic flux on the coverslip agreed well with the pattern of labelled DNA anchored on the coverslip. This result suggests that SQUID can be applied for the specific detection of DNA molecules, especially for the detection of DNA chips.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

The analysis of biological molecules is based on separation and detection. The detection method for the separated molecules determines the total sensitivity of the analysis system. To improve the sensitivity, several methods have been introduced for the detection. The radioisotope labelling method is one of the most common methods [1]; however, it requires a special laboratory in order to confine the radioisotope. The fluorescence labelling method is very sensitive. It can detect single molecules; however, the quenching of the fluorescence dye inhibits observation over long periods of time [2]. The enzyme labelling method also achieves high sensitivity; however, the enzymes are sometimes unstable [3].

Recently a labelling method employing small magnetic particles has been introduced. SQUID has a significant potential to detect small amounts of these particles because of its high sensitivity to magnetic fields. The detection of small magnetic particles with a SQUID for immunoassay applications has been performed by several groups [4, 5]. Even if particles are made of iron oxide, if they become small enough they display superparamagnetic properties. Therefore, some form of magnetic field should be applied to the particles for detection because they have almost no permanent magnetic dipole at room temperature. Koetits *et al* [4] applied a pulse field to the particles and then measured the field decay from the particles in the range of milliseconds. Enpuku *et al* [5] measured the field from the particles under a dc magnetic field. We measured the field from the particles under an ac magnetic field.

Recently a highly integrated DNA chip technique has been developed. Because the DNA chip loads enormous numbers of different DNA probes which correspond to genetic disease or personal properties, those have been applied to the analysis of the polymorphism of the human genome. We have developed a detection system and a sample preparation method to apply a high-T_c SQUID sensor for DNA chip detection.

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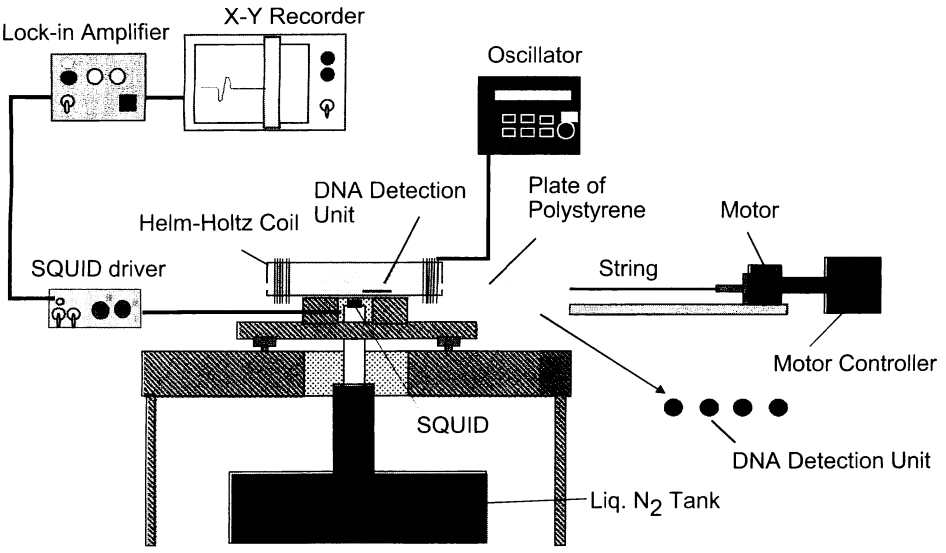

Figure 1. A schematic drawing of the high-T_c SQUID detection system. The magnetic field generated from the coils was modulated by a 100 Hz signal. A motor-driven string was employed to convey the coverslip into the coils.

2. Detection system

A schematic diagram of the system is shown in figure 1. The SQUID is made of $Y_1Ba_2Cu_3O_{7-y}$ thin film and was initially fabricated by the Sumitomo Electric Industry and then 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 × 1.0 mm^2 and the effective area is 0.068 mm^2. The SQUID was operated in a flux-locked loop with a flux modulation frequency of 100 kHz. 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 100 Hz was directed to the coils; the magnetic field generated by 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 was homemade 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 τ = 0.33 s). Since the rolloff provides sensitivity to noise only within 3 Hz 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 crucial in order to obtain good resolution in the system. In this scheme, as with signal averaging, the effect of the modulation is to centre the signal at the modulation frequency of 100 Hz, rather than at dc, in order to avoid $1/f$ noise, which occurs usually in the range from dc to 1 Hz. The two identical 1000 turn wound coils were spread apart by a distance of 50 mm.

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.

The system performance was evaluated. In this evaluation, the coverslip was replaced with a fine tube made of fluorocarbon. The details have been described elsewhere [12]. A suspension of the ultra-small particles supplied by Meito Sangyo Co., Ltd, was used as the magnetic particles. Similar particles were applied as a magnetic resonance imaging (MRI) contrast agent. The core of the particles was iron oxide Fe_3O_4 (magnetite) and they were coated with an alkali-treated dextran. The average core diameter was 11 nm, which was measured from the x-ray diffraction pattern using Scherrer's equation [9]. The particles had superparamagnetic properties. The particles were supplied as a suspension that contained 5.9 mg ml^{-1} of iron. If we suppose 5.2 g cm^{-3} as the specific gravity of the core, we can estimate the weight of the mono particle as 3.6 × 10^{-18} g and the total number of particles in the original suspension as 1.5 × 10^{16} ml^{-1}. The original suspension was diluted with deionized water to the desired concentrations.

We performed all of the measurements in a magnetically shielded room with a shielding factor of −50 dB at 0.1 Hz. The suspension was driven to the nearby high-T_c SQUID in a fine tube with a scan speed of 0.33–1.1 mm s^{-1} under an ac magnetic field of 9 × 10^{-5} to 4 × 10^{-4} T (peak-to-peak value). Figure 2 shows the typical output signal of the lock-in amplifier. In this experiment, the suspension containing 65 μg of iron extended by about 8 mm in the tube. The observed peak width was distributed for about 24–7.2 s which agreed well with the scan speed. In this measurement the distance from the SQUID to the specimen was 10 mm. Each positive and negative peak was observed when each edge of the suspension specimen passed over the centre of the SQUID. This means that each particle was homogeneously distributed in the suspension and that all of the particles behaved as one large dipole, whose length was 8 mm in our experiment.

Hereafter we define each difference of the negative and positive peaks as the SQUID signal. Measurements of magnetic fields were performed from prepared suspensions at different distances from the SQUID to the specimen. All of the conditions except for the concentration were the same

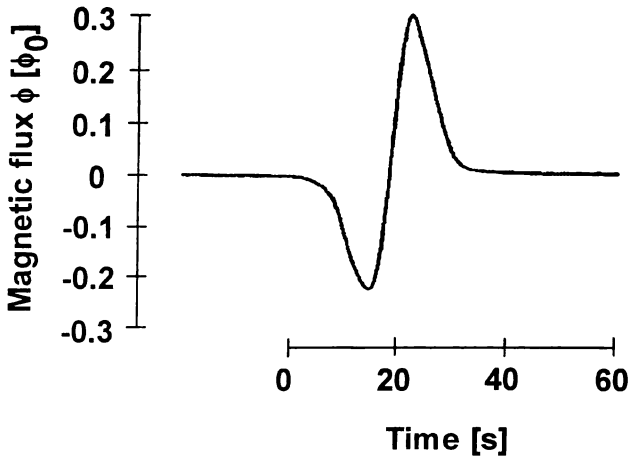

Figure 2. Typical output signal of the lock-in amplifier. The length of fluid in the tube was about 8 mm, this corresponds to the weight of iron in the fluid which was measured to be 65 μg. The distance from the SQUID to the specimen was 10 mm. Each positive and negative peak was observed when each edge of the fluid specimen passed over the centre of the SQUID.

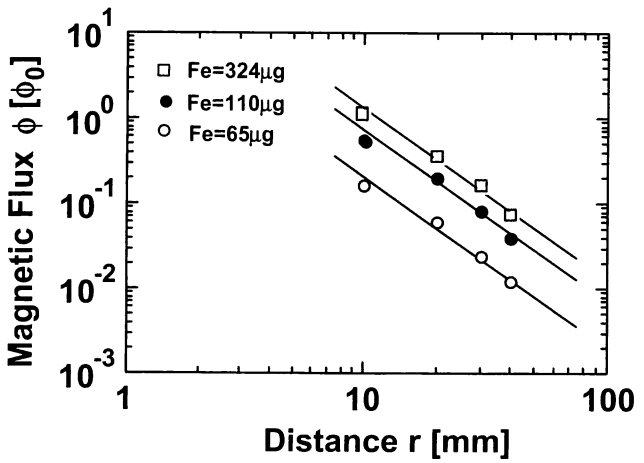

Figure 3. Magnetic signal intensity versus distances from the SQUID to the specimen. The fields were inversely proportional to the distance in power.

as in the previous experiment [12]. Figure 3 shows the results. Following the theory of classical electrodynamics [13], a field at a point from a dipole decreases as $1/r^3$, when $r \gg \lambda$, where r is the distance from the point to the dipole and λ is the length of the dipole. However, the fields were inversely proportional to the distance in power, because of the non-uniformity of the magnetic field around the sample. We investigated the detectable weight of the iron. The SQUID signal was proportional to the weight of the iron in the suspension. At a distance of 1 mm, the minimum detectable weight was 360 pg.

3. Preparation and measurement of coverslips with DNA

A DNA chip can detect the polymorphism of human DNA by detecting the mismatch. To detect DNA on the chip, both terminals of the DNA should be modified with different labels, because one label anchors the DNA on the DNA chip, the other label connects magnetic particles. We modified one

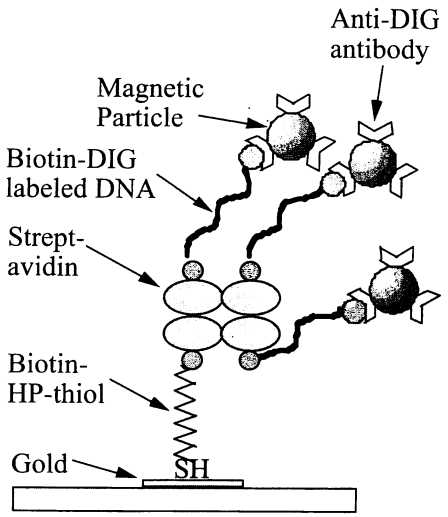

Figure 4. A schematic drawing of the anchoring of the DNA. Both terminuses of the DNA were labelled with biotin and DIG. The DNA was anchored on a gold layer using a biotin–avidin complex. Magnetic particles were bound using an anti-DIG antibody.

terminal with biotin and the other with digoxigenin (DIG). This labelling was carried out using PCR (polymerase chain reaction) with two different primers labelled with biotin and DIG [10].

The labelled DNA was anchored on a gold layer patterned on a glass coverslip as shown in figure 4. First biotin was fixed on a gold layer through the thiol-group, then avidin was fixed through the avidin–biotin complex [11]. Because avidin has four sites for biotin binding, the labelled DNA was anchored through the remaining biotin binding sites of the avidin. The other label, DIG, was used for labelling with small magnetic particles. DIG was bound to the magnetic particles through the anti-DIG antibody. The prepared coverslip was scanned by the high-T_c SQUID detection system.

The coverslip loading probe DNA was confirmed by fluorescent visualization of the DNA. Figure 5 shows the image of the fluorescent DNA obtained using a fluorescence microscope. The bright spots in figure 5 correspond to anchored DNAs. This result indicated that the probe DNA was anchored tightly and localized on the gold coated surface.

A series of DNA samples was prepared on a glass coverslip as we could not evaluate the amount of DNA due to difficulties in the measurement of the binding efficiency. Therefore the series was represented in arbitrary units as 1, $\frac{1}{2}$, $\frac{1}{4}$ and $\frac{1}{8}$ of DNA. The coverslip was scanned by the high-T_c SQUID system. Figure 6 shows the result of scanning the coverslip. The pattern of magnetic flux detected by the high-T_c SQUID agreed with that of the loaded DNA.

4. Discussion

Evaluation of the system performance demonstrated that the system detected 3.6×10^{-10} g of iron in the magnetic particles, which corresponded to 10^8 particles because each particle contains 3.6×10^{-18} g of iron. Because one DNA molecule connected to one particle, the detection limit of single stranded DNA (ssDNA) can be estimated using the molecular weight of ssDNA, which is a product of 330 (average molecular weight

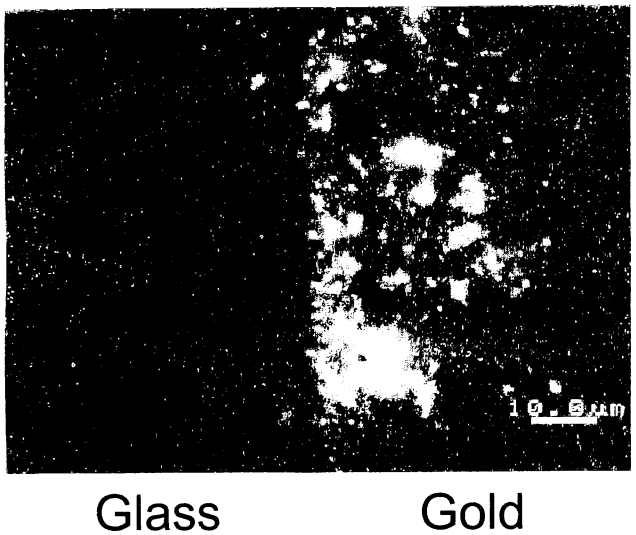

Figure 5. Anchored DNA on the gold coated surface. The anchored DNA was visualized by fluorescent dye, YOYO-1, and observed by a microscope. This figure shows the boundary between the glass (left) and gold coated surface (right).

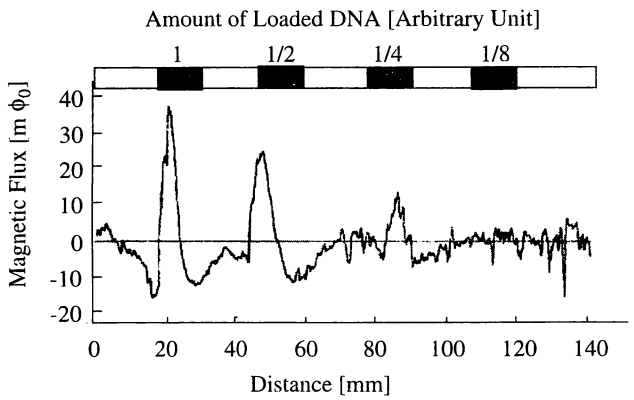

Figure 6. Scanning of a DNA loaded coverslip. The series of DNA samples (1, $\frac{1}{2}$, $\frac{1}{4}$ and $\frac{1}{8}$) was prepared on a coverslip and scanned by a high-T_c SQUID detection system.

of the nucleotide) and the length of ssDNA in nucleotides. Therefore, 1000 nucleotides of ssDNA is 3.3×10^5 in molecular weight, which corresponds to 5.5×10^{-19} g. The detection limit in ssDNA can be obtained as the product of the number of particles (10^8) and mass of 1000 nucleotides of ssDNA (5.5×10^{-19} g), therefore calculation gives 55 pg of 1000 nucleotides of ssDNA. The result suggests this system can be applied to the detection of the DNA chip.

5. Conclusion

A system for the detection of biological molecules using a high-T_c SQUID magnetometer has been 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 [12]. This system permits long periods of observation of the molecules and can suppress interference due to enzymes in the samples. These advantages may lead to a new detection method that will solve the problems with conventional detection methods. The system succeeded in detecting DNA molecules arranged on a glass coverslip.

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