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# Feature Article: SQUID Applications - Medical Applications <br> - Development of Highly-Sensitive Biological Immunoassays utilizing Magnetic Markers 

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Biological immunoassays are widely used medical tests to detect protein and pathogenic bacteria biomaterials originating from various types of diseases. The author and his group have investigated magnetic markers and high temperature SQUID magnetic sensors in the development of magnetic immunoassays. Advancements in low-noise SQUID sensor technology together with a selection of magnetic markers suitable for immunoassay studies have proved successful in significantly improving the sensitivity of magnetic immunoassays.

The biological immunoassay we developed requires a SQUID sensor that exhibits low frequency noise characteristics since the frequency of the measured signal is around 10 Hz . Figure 1 (a) shows the flux noise spectrum of a SQUID sensor at 77 K , developed jointly with the Superconductivity Research Laboratory (SRL). A comparison between the results using DC bias or AC bias to drive the SQUID was performed. As shown in the figure, the flux noise is given by $S_{\Phi}{ }^{1 / 2}=8 \mu \Phi_{0} / \mathrm{Hz}^{1 / 2}$ at high frequencies exceeding 100 Hz . A DC-biased SQUID has increased noise at low frequencies, given by 1/f. To the contrary, an AC-biased SQUID avoids low frequency noise increases, instead, having flux noise characteristics given by $S_{\Phi}{ }^{1 / 2}=15 \mu \Phi_{0} / \mathrm{Hz}^{1 / 2}$ at $\mathrm{f}=1 \mathrm{~Hz}$. Thus, a SQUID sensor exhibiting reduced low frequency noise has been successfully developed.

Magnetic markers (FG beads) manufactured by Tamagawa Seiki Co., Ltd. have been employed. These markers are in fact magnetic nanoparticles $\left(\mathrm{Fe}_{3} \mathrm{O}_{4}\right)$ around 40 nm in size and coated with glycidyl methacrylate (GMA) polymer. The magnetic nanoparticles agglomerate forming a polymer with an approximate diameter of 270 nm .


Fig. 1 (a) Flux noise spectra of high temperature SQUID sensor, (b) Detection of magnetic marker utilizing SQUID. The relationship between the numbers of magnetic markers $\mathrm{N}_{\mathrm{m}}$ and the signal detected.

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Figure 1(b) shows the results of a magnetic signal detected from the magnetic markers acquired using a SQUID sensor. The horizontal axis shows the number $\mathrm{N}_{\mathrm{m}}$ of magnetic markers, whilst the vertical axis shows the signal flux detected by the SQUID. As the peak-to-peak SQUID noise is approximately $0.05 \mathrm{~m} \Phi_{0}$, it is understood from the results in Figure 1(b) that around $5 \times 10^{3}$ magnetic markers are detectable. Assuming that just one magnetic marker is bound with a single antigen, the results imply that it is possible to detect $5 \times 10^{3}$ antigens.

Detection trials of proteins (bioten) using these magnetic markers involved the following. Firstly, as shown in Figure 2(a), biotin was fixed to the surface of the polymer beads, or more specifically, 2,000 biotins fixed per one polymer bead. Next, the magnetic markers were conjugated by avidin, which was added into the liquid sample. The coupling between biotin and avidin produces magnetic markers that are partially bound to the polymer beads, and become bound markers. The remaining magnetic markers are free markers and both types are mixed in the liquid. It is possible to magnetically distinguish between the two sets of markers utilizing the Brownian magnetic relaxation of free markers and without the need for a washing process step.

Figure 2(b) shows the relationship between the numbers of biotin N bound to polymer beads with the associated signal flux. There is a correlation between N and the signal flux as shown in the figure, suggesting that immunoassays analysis can be performed precisely without the need of the washing process step. The sensitivity shows a successful detection of $2 \times 10^{4}$ biotin. The liquid volume for this test was $35 \mu /$. When described in molar concentration, the detection ability is $9.5 \times 10^{-19} \mathrm{~mol} / \mathrm{ml}$, which is sufficient to ensure very high sensitivity detection. One of the advantages of the magnetic method is to eliminate the washing process step necessary to separate free markers in the immunoassays. The aforementioned results indicate the possibility of high sensitivity immunoassays utilizing the method we developed. Further high sensitivity testing is anticipated when the performance of magnetic markers is enhanced.

(a)


Fig. 2 Immunoassays using the magnetic marker method. (a) Detection of proteins (biotin) using bound polymer beads, (b) The relationship between the number of biotin N and their associated signal flux

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