

## Sentinel Lymph Node Biopsy by High-T<sub>c</sub> SQUID Gradiometer

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### Abstract

We have proposed Lymph-node detection system using a high T<sub>c</sub> SQUID gradiometer and ultra-small particles. A rat was injected with a mixture of water diluted superparamagnetic particles. The lymph nodes containing particles were then extracted from the rat. The lymph node samples were measured by a SQUID gradiometer system. We have successfully measured the signal from the lymph node.

**KEYWORDS:** High-T<sub>c</sub>, SQUID, Superconductor, lymph node, biopsy, small particles

### 1. Introduction

We have been proposing the application of a high-T<sub>c</sub> superconducting quantum interference device (SQUID) for a sentinel node biopsy, which has been a newly developed surgical technology. Axillary lymph-node dissection is an important procedure in the surgical treatment of breast cancer. However, in the early diagnosis stage, the number of dissections in which axillary nodes are free of disease is apt to be increased. These treatments lead to some problems such as a lymph edema and a sensory neuropathy in the patient. The sentinel node biopsy is a kind of test to investigate whether the sentinel node, which initially receives malignant cells from a breast carcinoma is disease-free or not. If the sentinel node is free of disease, you can leave the rest of the lymph-nodes because of no concern for progression. This biopsy is based on the hypothesis that if the first lymph node (sentinel node) is free of disease, the second and the rest of the nodes must be negative. Two methods which detect the sentinel node have been developed and reported to date [1]-[3]. One is a kind of radio guide, which uses a gamma detector and a radio isotope such as technetium labeled sulfur colloid. After injecting the isotope into a breast lesion, the sentinel lymph node will be identified by the gamma detector. Then the sentinel node is excised and examined. In this method the sentinel lymph-node is successfully identified with 94.4% accuracy [1]. Though the predictability of this method is extremely high, radiation exposure is inevitable for medical staff. The other method uses a blue dye; a surgeon identifies the sentinel lymph-node with his naked eye. With this method the predictability is still 70% accurate [2].

Therefore we propose a localization system combined with a high sensitivity superconducting quantum interference device (SQUID) gradiometer and

ultra-small iron oxide particles. The system we are proposing is shown in Fig. 1. The particles are injected into the breast; and the high-T<sub>c</sub> SQUID is used as a sensing detector for the particles. This method has some advantages: no radiation exposure and an accurate identification because of the visible color of the particles themselves. For this application, the SQUID magnetic sensor should identify the location of the small quantity of particles under the sensor at a distance of several centimeters. Even if the particles are made of iron oxide, if their size becomes too small, 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. Koetitz et.al. applied a pulse field to the particles and then measured the field decay from the particles in the range of ms [4]. Empuku et.al. measured the field from the particles under a DC magnetic field [5], [6]. We have already reported the results of preliminary study using particles dispersed liquid in a tube by an AC magnetic field [7].

In this paper, we describe the results of the detection of pseudo-lymph nodes made of balloon, and real lymph nodes extracted from a rat by a SQUID gradiometer.

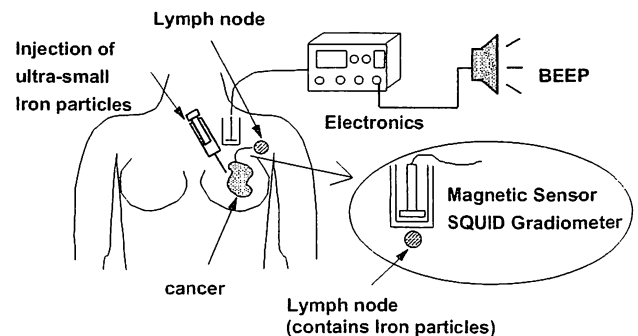


Fig. 1 Diagram of the proposed system.

### 2. Experimental Setup

The schematic diagram of the system is shown in Fig. 2. The SQUID gradiometer is made of Y<sub>1</sub>Ba<sub>2</sub>Cu<sub>3</sub>O<sub>7-y</sub> thin film. The junctions utilized in the SQUID are of the step-edge type. The gradiometer is a planer type which baseline is 3.6 mm. The gradiometer was operated in a

flux-locked loop with a flux modulation frequency of 256 kHz. The magnetic flux noise in the white noise region was about  $20 - 30 \mu\phi_0/\text{Hz}^{1/2}$ .

The cryostat was specially designed for a SQUID microscope. The SQUID Sensor was located inside a vacuum and separated by a  $500\mu\text{m}$  thick quartz window. A more detailed description can be found elsewhere [8]. A 750 turns wound coil for magnetization was mounted just above the SQUID microscope [9], [10].

A balloon sample on a polyethylene sheet was drawn by an induction motor. A dc current was directed to the coils. The magnetic field generated from the coil can magnetize a sample above the coil. The SQUID gradiometer position was carefully adjusted before measurement, so that the SQUID output signal without particles became zero. After adjusting the SQUID position, the sample was moved with the scan speed of  $0.3-1.0 \text{ mm/sec}$  under a dc magnetic field of  $4 \times 10^{-4} - 8 \times 10^{-4} \text{ T}$ .

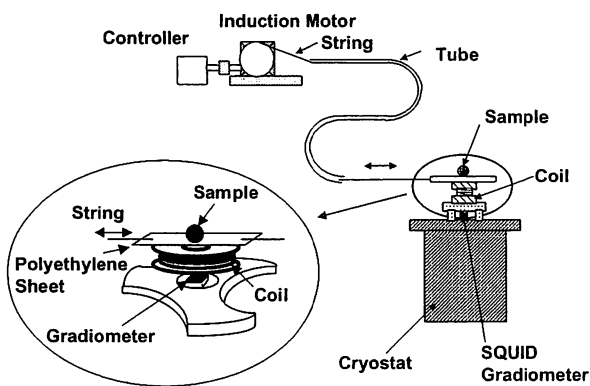


Fig.2 Schematic diagram of the measurement of the samples.

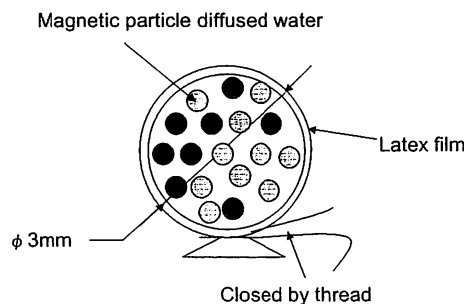
### 3. Samples

We used ultra-small particles from Meito Sangyo Co., Ltd. Similar particles are used as a magnetic resonance imaging (MRI) contrast agent. The core of the particle is iron oxide  $\text{Fe}_3\text{O}_4$  (magnetite) which is coated with an alkali-treated dextran. The average core diameter was  $11\text{nm}$ . The particles had superparamagnetic properties. The particles were supplied in the form of an aqueous magnetic fluid. The original fluid contained  $5.9 \text{ mg/ml}$  of iron. If we suppose  $5.2 \text{ g/cm}^3$  as the specific gravity of the core, we can estimate the weight of the mono-particle as  $3.6 \times 10^{-18} \text{ g}$  and the total number of particles in the original solution as  $1.5 \times 10^{16}/\text{ml}$ . The original fluid was diluted with distilled water to have the desired concentrations. Then the diluted fluid was wrapped with a latex film as shown in Fig.3. The outer dimension of the balloon is  $3 \text{ mm}$  in diameter. We used the balloon sample as a pseudo lymph node through our experiment.

### 4. Animals

Male Wister Shionogi rats (WS; Shionogi Aburabi Laboratory, Shiga, Japan, 10 weeks old) weighting 290-320 g were used in the following experiments. The rats were kept in a temperature-controlled room with a 12-hour light-dark cycle and acclimated for at least 7 days before use. All animals had free access to water and standard laboratory diet (Oriental Yeast Co, Ltd, Tokyo, Japan). All procedures were performed under pentobarbital anesthesia ( $50\text{mg/kg}$ , i.p.), and all experiments were carried out in compliance with guidelines on the care and use of laboratory animals from Osaka University.

The hind legs of a rat were injected with a mixture of water diluted particles and lymphazurin which was used as a color dye for a human lymph node operation. The total volume was  $400\mu\text{l}$  and the iron content of the mixture was  $0.56\mu\text{g}/\mu\text{l}$ . After 5 minutes, the particle contained lymph nodes were then extracted from the rat. The samples initially underwent fixation in formaldehyde to prevent structure. Following fixation, the samples were dehydrated in graded ethanol-water mixtures, and



embedded in paraffin wax.

Fig.3 Schematic drawing of a pseudo lymph node sample [11].

### 5. Results and discussion

All of the measurements were performed in a magnetically shielded room with a shielding factor of  $-50 \text{ dB}$  at  $0.1 \text{ Hz}$ . Fig. 4 shows the typical output signal of the gradiometer when the spherical sample passed above the coil. The weight of iron in the fluid was calculated as  $25\mu\text{g}$ . The measurement was performed under the dc applied magnetic field of  $6.4 \times 10^{-4} \text{ T}$ . In this measurement the distance from the sensor to the specimen was  $6\text{mm}$ . Large positive and negative peaks are observed. Each positive and negative peak was recorded when the sample was above each pickup loop of the gradiometer. We define peak to peak voltage as a output signal. It is correspond to the weight of the iron.

We investigated the detectable weights of the iron at the distance of 6mm. As shown in Fig.5, the SQUID signal was proportional to the weight of the iron in the fluid. Particles of 100ng in weight of iron could be detected with a spacing of 6mm.

Then lymph nodes retrieved from a rat were used as samples. The dimension of the sample is about 6mm x 3mm x 1mm (Length x Width x Thickness). Measurement condition was the same as the pseudo samples. Fig.6 shows the applied magnetic field dependence of the signal. The signal intensity is 31 mφ<sub>0</sub> at magnetic field of 6 x 10<sup>-4</sup> T. The iron content of the lymph node sample can be calculated as 100 ng from the results of the pseudo sample shown in Fig.5. This performance is good enough to apply this system to the real sentinel lymph node biopsy.

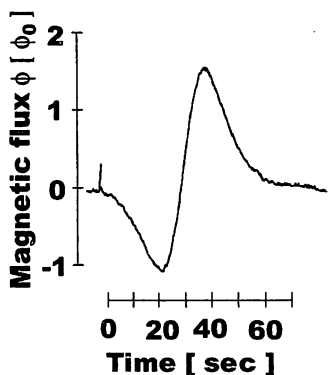


Fig.4 Typical output signal when a spherical sample passed above the gradiometer. Large positive and negative peaks are observed.

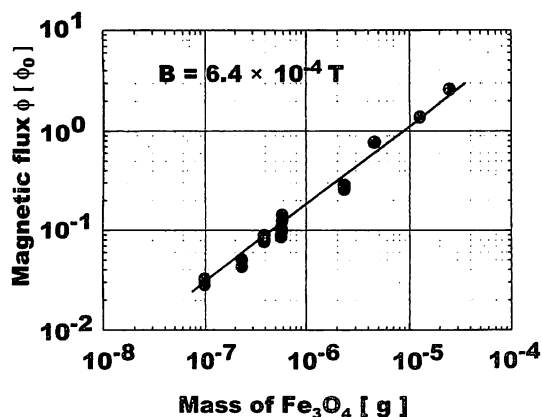


Fig.5 Magnetic signal intensity from pseudo lymph node sample vs. weight of Fe in particle.

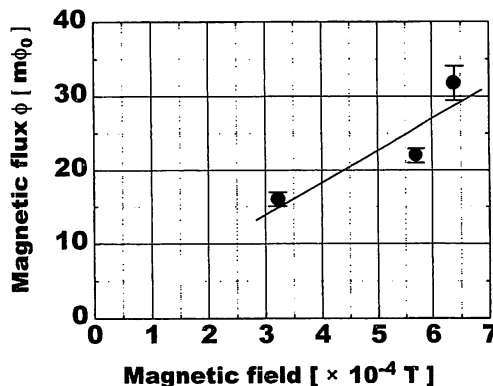


Fig.6 Applied magnetic field dependence of the signal. The sample was the rat's lymph node containing superparamagnetic nano-particles.

### 6. Conclusion

We have demonstrated the possibility of sentinel lymph node biopsy using a high-Tc SQUID gradiometer. Pseudo spherical lymph nodes and real rat's lymph nodes were used as samples. The ultra-small iron oxide particles of 100ng in weight of iron could be detected with a spacing of 6mm under dc magnetic field. Rat's lymph nodes containing iron oxide particles were measured by the same system. We could successfully measure the signal from the rat's lymph node.

The resolutions are good enough to apply the technology for a Sentinel-node biopsy and a lymphatic mapping. Next step should be a in vivo experiment.

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