Detection of Lymph Nodes of Rat by High T_c SQUID

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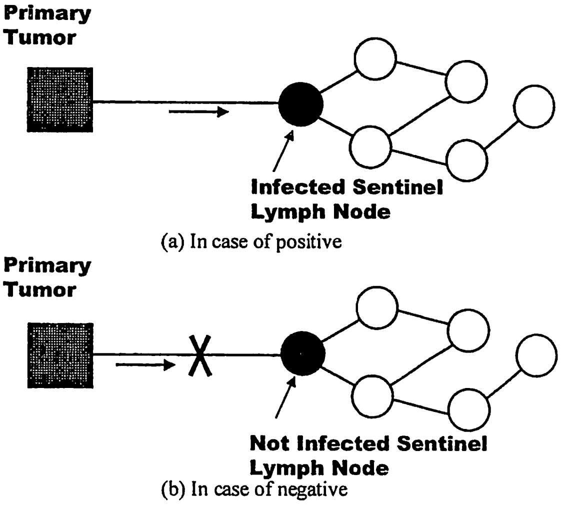
Abstract We have proposed a Lymph-node detection system using a high Tc SQUID gradiometer and ultra-small particles. A rat was injected with a mixture of water diluted superparamagnetic particles. The lymph nodes containing particles were then extracted from the rat. The lymph node samples were measured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

Keyword SQUID, nano, biopsy, breast cancer, lymph node

1. Introduction

We have proposed the application of a high-T_c superconducting quantum interference device (SQUID) for sentinel node biopsy, which is 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 tends to be high. These treatments lead to problems such as a lymph edema and a sensory neuropathy in the patient. Sentinel node biopsy is used 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, it is unnecessary to remove the rest of the lymph-nodes because of no concern for the progression. The principle is shown in figure 1. An infected area (primary tumor) is connected with axillary lymph nodes. This biopsy is based on the hypothesis that if the first lymph node (sentinel node: shown as a solid circle in the figure) is free of disease, the second and the rest of the nodes must be also negative. In case of positive (a), all of the lymph nodes should be dissected because of the possibility of the progression in the future. In case of negative (b), you can preserve the rest of the lymph nodes. 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 the 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 particles are injected into the breast; and the high-T_c SQUID is used as a sensing detector for the particles. This method has some advantage: no radiation exposure and an accurate identification because of the visible color of the particles themselves. A surgeon identifies colored lymph tube and finds the target sentinel lymph node easily. 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. Therefo



(a) In case of positive

(b) In case of negative

Fig. 1. Principle of the sentinel lymph node biopsy. Sentinel lymph n should be identified by a sensor and remove for the biopsy. After the bio the strategy will be decided. . In case of positive (a), all of the lymph nc should be dissected because of the possibility of the progression in future. In case of negative (b), you can preserve the rest of the lymph nc [4].

re, 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 [5]. Empuku et.al. measured the field from the particles under a DC magnetic field [6], [7]. We have already reported the results of a preliminary study using particles dispersed in a liquid contained tube by an AC magnetic field [8].

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

1. Experimental Setup

The SQUID gradiometer is made of $Y_1Ba_2Cu_3O_{7-y}$ 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 $20 - 30\ \mu\phi_0/Hz^{1/2}$. The measured effective area as a magnetometer was $0.13mm^2$. This value was measured by cutting one pickup loop of the two.

The cryostat was specially designed for a SQUID microscope. The SQUID sensor was located inside a vacuum and separated by a 500µm thick quartz window. A more detailed description can be found elsewhere [9]. A 750 turns wound coil for magnetization was mounted just above the SQUID microscope [10]-[12]. This coil can generate magnetic field of up to 8×10^{-4} T.

All of the experiments were performed inside a magnetically shielded room (MSR), with a shielding factor of –50dB at DC. The schematic diagram of the system is shown in Fig. 2. A balloon sample on a polyethylene sheet was drawn by an induction motor installed outside the MSR. A 750 turns wound coil was on the cryostat. The bore size was 6mm in diameter. A dc current was directed to the coil. The magnetic field generated from the coil can magnetize a sample above the coil. The distance between the sample and the SQUID was 7mm. The SQUID gradiometer position was carefully adjusted before measurement, so that the SQUID output signal without particles was almost zero. After adjusting the SQUID position, the sample was moved with a scan speed of 0.3-1.0 mm/sec under a dc magnetic field of $4 \times 10^{-4} - 8 \times 10^{-4}$ T. We note that the both of white and low frequency noise remain constant for th magnetic field in this range.

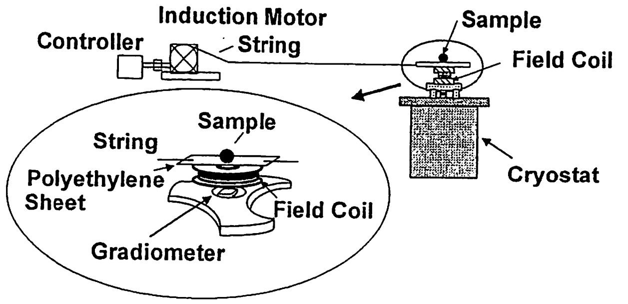


Fig.2. Schematic diagram of the measurement system. A 750 turns wound coil was on the cryostat. The bore size was 6mm in diameter. The SQUID gradiometer position was carefully adjusted before measurement, so that the SQUID output signal without particles became almost zero. Then the sample was moved under a dc magnetic field of $4 \times 10^{-4} - 8 \times 10^{-4}$ T.

We used ultra-small particles from Meito Sangyo Co., Ltd Similar particles are used as a magnetic resonance imagin (MRI) contrast agent. The core of the particle is iron oxid Fe_3O_4 (magnetite) which is coated with an alkali-treated dextra The average core diameter was 11nm. The particles ha superparamagnetic properties. The particles were supplied i the form of an aqueous magnetic fluid. The original flui contained 5.9 mg/ml of iron. If we suppose $5.2\ g/cm^3$ as th specific gravity of the core, we can estimate the weight of th mono-particle as $3.6x10^{-18}$ g and the total number of particles i the original solution as $1.5x10^{16}$/ml. The original fluid wa diluted with distilled water to have the desired concentrations Then the diluted fluid was wrapped with a latex film. The oute dimension of the balloon is 3 mm in diameter. We used th balloon sample as a pseudo lymph node during our experiment

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

The hind legs of a rat were injected with a mixture of wate diluted particles and lymphazurin which was used as a color dye for a human lymph node operation. The total volume was 200µl and the iron content of the mixture was 24.5µg/µl. Thus a total mass of 4.9 mg iron was injected to the rat. After 5 minutes, the particle containing lymph nodes were then extracted from the rat (Fig.3). The samples initially underwent fixation in formaldehyde to preserve structure. Following fixation, the samples were dehydrated in graded ethanol-water mixtures, and embedded in paraffin wax.

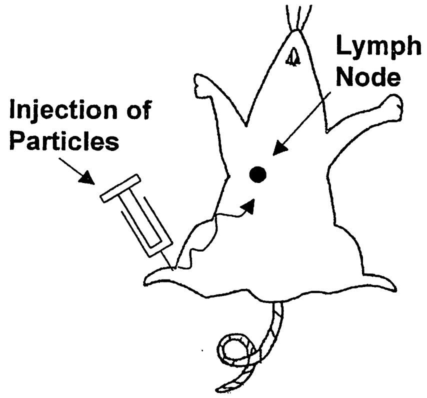


Fig.3. Schematic drawing of the preparation of lymph nodes of rat. The hind legs of a rat were injected with a mixture of water diluted particles and lymphazurin which was used as a color dye for a human lymph node operation. After 5 minutes, the particle contained lymph nodes were then extracted from the rat.

2. Results and discussion

(1) Pseudo Lymph Node

All of the measurements were performed in a magnetically shielded room with a shielding factor of –50 dB at 0.1 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 25μg. The measurement was performed under the dc applied magnetic field of 6.4×10^{-4} T. In this measurement the distance from the sensor to the specimen was 6mm. 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 corresponds to the weight of the iron.

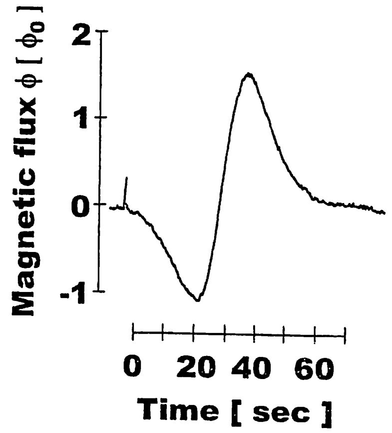


Fig. 4. Typical output signal when a spherical sample passed above the gradiometer. Large positive and negative peaks are observed.

We investigated the detectable weight of the iron at a 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.

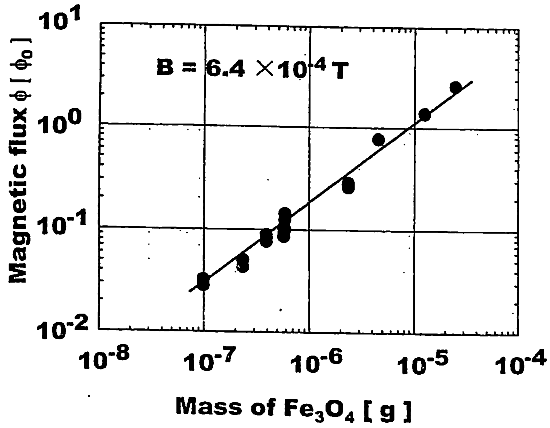


Fig.5. Magnetic signal intensity from pseudo lymph node sample vs. weight of Fe in particle. Particles of 100ng in weight of iron could be detected with a spacing of 6mm

(2) Rat Lymph Node

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). The measurement conditions were the same as for the pseudo samples. Fig.6 shows the dependence of the signal on applied magnetic field. The signal intensity was fairly large and 1250 mϕ_0 at a magnetic field of 6.4×10^{-4} T. The iron content of the lymph node sample can be calculated as 10 μg from the results of the pseudo sample shown in Fig.5. This value correspond to 0.2% of the injected iron. Although it is difficult to estimate the accumulated weight of iron particles for the human case, we think it must be more than 10μg because the volume of the injection must be 20 times larger than that of the rat. This performance is good enough to apply this system to the real sentinel lymph node biopsy. The effective area of our gradiometer is 0.13 mm^2. If supposing the magnetic field from the sample is uniform, then ϕ_0 corresponds to about 15 nT. This value is large enough for a high Tc SQUID and also might be detected by other sensors e.g. a magnetoresistive sensor or a flux gate sensor. A test for a large animal should be performed before applying for human to estimate signal intensity from the lymph. One should also know that more sensitivity needs for the real SQUID biopsy with a distance of several centimeters. We can determine if the SQUID is the only sensor which can be used for sentinel node biopsy in the near future.

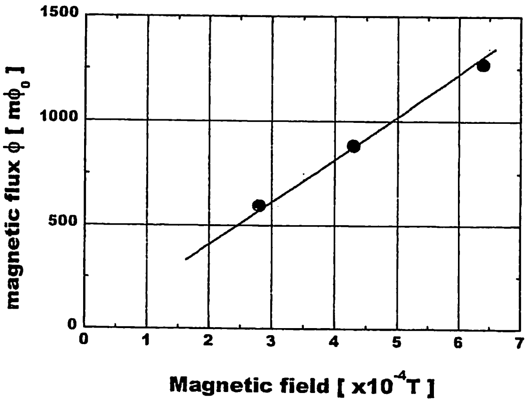


Fig6. Applied magnetic field dependence of the signal. The sample was the rat's lymph node containing superparamagnetic nano-particles. The signal intensity was fairly large and 1250 mϕ_0 at magnetic field of 6.4×10^{-4} T.

3. 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 estimated weight of the lymph node was 10μg. The resolutions are good enough to apply the technology for a Sentinel-node biopsy and a lymphatic mapping.

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