Research articles

Separation of excitation and detection coils for in vivo detection of superparamagnetic iron oxide nanoparticles

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A R T I C L E   I N F O

Keywords:
- Differential magnetometry
- Sentinel node biopsy
- Superparamagnetic iron oxide nanoparticles (SPIONs)
- Nonlinear susceptibility

A B S T R A C T

A novel probe for laparoscopic in vivo detection of superparamagnetic iron oxide nanoparticles (SPIONs) has been developed. The main application for in vivo detection of SPIONs in our research group aims at sentinel node biopsy. This is a method to determine if a tumor has spread through the body, which helps to improve cancer patient care. The method we use to selectively detect SPIONs is Differential Magnetometry (DiMag). DiMag makes use of small magnetic field strengths in the mT range. For DiMag, a handheld probe is used that contains excitation and detection coils. However, depth sensitivity of a handheld probe is restricted by the diameter of the coils. Therefore, excitation and detection coils are separated in our novel probe. As a result, excitation coils can be made large and placed underneath a patient to generate a sufficiently large volume for the excitation field. Detection coils are made small enough to be used in laparoscopic surgery. The main challenge of this setup is movement of detection coils with respect to excitation coils. Consequently, the detector signal is obscured by the excitation field, making it impossible to measure the tiny magnetic signature from SPIONs. To measure SPIONs, active compensation is used, which is a way to cancel the excitation field seen by the detection coils. SPIONs were measured in various amounts and at various distances from the excitation coils. Furthermore, SPIONs were measured in proximity to a surgical steel retractor, and 3L water. It is shown that small amounts of SPIONs (down to 25 μg Fe) can be measured, and SPIONs can be measured up to 20 cm from the top of the excitation coil. Also, surgical steel, and diamagnetism of water – and thus of tissue – have minor influence on DiMag measurements. In conclusion, these results make this novel probe geometry combined with DiMag promising for laparoscopic sentinel node biopsy.

1. Introduction

Sentinel node biopsy (SNB) is a procedure to determine the lymph node status of cancer patients [1]. As a result, it can be determined if the tumor has spread through the body and consequently patient care will be improved. In this paper, a novel probe for laparoscopic SNB is presented, as shown in Fig. 1. Using such a minimally invasive approach results in improved short-term outcome for infections, hospital stay and quality of life compared to open surgery [2]. Laparoscopic SNB can be applied for many types of tumors, including prostate [3], bladder [4], esophageal [5] and gynecologic [6] cancers.

During SNB, a tracer is injected close to the tumor. This tracer will follow the natural path through the lymphatic system via passive mechanical transport and it will accumulate in the first nodes it encounters, namely the sentinel nodes. The next step in SNB is identification of the sentinel nodes using a dedicated probe. Finally, both the primary tumor and sentinel nodes are surgically removed.

Various types of tracers can be used for SNB. Traditionally, a radioisotope tracer is used in combination with blue dye. However, this has several disadvantages, including logistical difficulties [3]. A promising alternative is a fluorescent tracer, which is frequently used in laparoscopic surgery [7,8]. The most important advantages of this tracer are that it can be visualized using a standard laparoscopic camera and it is possible to map lymphatic drainage pathways in real time. However, the main disadvantages are its limited depth sensitivity (< 10 mm) and rapid distribution (fluorescent tracer does not get trapped in sentinel nodes), giving the surgeon limited time to find sentinel nodes [3,7,9].

Another promising tracer for SNB are superparamagnetic iron oxide nanoparticles (SPIONs). This magnetic tracer has many advantages over a radioactive one, since it has a long shelf life and no strict regulations [10]. The main advantage of a magnetic tracer over a fluorescent one is that SPIONs get trapped inside sentinel nodes, giving the surgeon more time to find them. Furthermore, we expect that eventually depth
sensitivity will be improved with our novel laparoscopic probe.

Sentinel nodes have a mean depth of 4 cm (1.5–8.5 cm) in breast cancer patients [11]. Approximately 0.3% of the injection amount of SPIONs ends up in sentinel nodes [12,13]. With a standard injection dose, it was found that a sentinel node contains 140 ± 80 μg Fe [12].

To detect SPIONs in vivo, several handheld probes were developed for open surgery. These probes make use of AC magnetometry [14], magnetic tunnelling junction [15], a combination of a permanent magnet and Hall sensor [12], or a fundamental mode orthogonal fluxgate gradiometer [16]. However, the main disadvantage all these probes share is their sensitivity to both surgical steel and diamagnetism of tissue. This sensitivity to diamagnetism limits depth sensitivity for low dose detection [17].

Differential Magnetometry (DiffMag) does not suffer from this disadvantage. DiffMag is a method that makes use of the nonlinear magnetic properties of SPIONs, which enables selective detection [18]. To detect SPIONs in vivo, a handheld probe was developed, which contains excitation and detection coils [19]. However, this first handheld probe has limited depth sensitivity. Depth sensitivity depends on the diameter of the coils. In laparoscopic surgery, the diameter of the probe is limited, because the probe has to fit through a standard laparoscopic trocar (12 mm). If the diameter of the handheld probe is decreased to 12 mm, depth sensitivity will decrease. As a result, it will be impossible to detect sentinel nodes that lie deeper in tissue, which is a prerequisite for SNB.

Our solution to improve depth sensitivity is mechanical separation of excitation and detection coils. In this way, the excitation coils can be made large to generate a sufficiently large volume for the excitation field. These large coils will be placed underneath the patient. The detection coils will be made small enough to fit through standard laparoscopic trocars and will be used as handheld probe.

The main challenge after separating excitation and detection coils is movement of the detection coils with respect to the excitation coils. As a result, the detection signal will be obscured by the excitation field and it becomes impossible to detect tiny magnetization of SPIONs. To solve this problem we make use of active compensation. In active compensation, extra field is coupled in, to cancel the measured excitation field. This leads to a balanced probe and SPIONs can be measured. A second goal of active compensation is to cancel the contribution of materials with a linear magnetic susceptibility in the mT field range, such as tissue and surgical steel.

Active compensation is only possible because we use DiffMag. In DiffMag, a combination of an AC field and DC offsets is used. When a DC offset is applied, the amplitude of the measured signal is lower compared to when no offset is applied due to nonlinearity of SPIONs. The difference in amplitude between blocks with and without DC offset is defined as DiffMag counts. This is a selective, quantitative measure for SPIONs. By coupling in extra field, as is done in active compensation, the amplitude of the measured signal will change, but the difference in amplitude remains the same. Therefore, distortions in balance of the probe do not influence DiffMag measurements.

However, in conventional AC magnetometry only an AC excitation field is used. In this case, the amplitude of the measured signal is indicative for the amount of SPIONs in proximity to the probe. As a result, the extra coupled field has exactly the same effect as measuring a lower quantity SPIONs, or measuring them further away from the probe. Therefore, it is impossible to distinguish the magnetization of SPIONs from distortions in balance of the probe.

The main reason to balance the probe with active compensation is to optimize amplification gain and stay in the sensitive region of the data acquisition system. The goal of this paper is to describe and demonstrate active compensation. Furthermore, the first static SPION measurements using our novel probe are shown. Finally, it is shown that measurements are not disturbed by surgical steel or diamagnetism of tissue.

2. Materials

In this paper, SHP-25 (Ocean Nanotech) particles were used. These are water soluble iron oxide nanoparticles. They have a single magnetite core with a diameter of 25 nm and a 4 nm thick amphiphilic polymer coating [20,21]. They were measured in their standard concentration of 5 mg(Fe)/mL.

This magnetite core – polymer shell structure is typical for SPIONs. A clinical tracer is for example Sienna+, a CE-marked magnetic tracer intended for sentinel node biopsy. This tracer also has a core–shell structure [14,22]. However, magnetic behavior of a monodisperse particle like SHP-25 is easier to predict, so we use this particle for developmental purposes.

3. Methods

3.1. Differential magnetometry

DiffMag is a method to selectively detect SPIONs in vivo, as previously described by Visscher et al. and Waanders et al. [18,19]. It combines a continuous alternating (AC) magnetic field that has a small amplitude with positive and negative DC offset fields, as shown in Fig. 2. As a result, every iteration of the excitation sequence consists of four blocks: no offset, positive offset, no offset, negative offset. Due to nonlinearity of SPIONs, the amplitude of the signal in a block with DC offset is lower compared to the signal in a block without DC offset. The difference in amplitude between these blocks is defined as DiffMag counts. This is a quantitative, selective measure for SPIONs.

3.2. Active compensation

Since the detection coils can move with respect to the excitation coils, their mutual inductance changes. As a result, the detection signal is obscured by the excitation field, making it impossible to detect tiny magnetization of SPIONs. Part of the excitation field is eliminated, because the detection coils are in a gradiometer configuration. However, to further optimize balance of the moving probe, active compensation is required. To achieve this, compensation coils are used, which are wound directly around the two detection coils. The phase and amplitude of the current that is sent through the compensation coils (and thus the magnetic field they produce) can be adjusted using two 10-bit digital potentiometers.

The induction voltage in the detection coils \(U_{det}\) is proportional to the time derivative of four contributions, as shown in the following equation:
In this equation $M_{\text{SPION}}$ is the nonlinear magnetization of SPIONs, $M_{\text{lin}}$ is the magnetization of materials with a linear susceptibility (for example, tissue and surgical steel), $H_{\text{exc}}$ is the excitation field strength and $H_{\text{comp}}$ is the compensation field strength. The goal of active compensation is to make $H_{\text{comp}}$ equal to $M_{\text{lin}} + H_{\text{exc}}$.

The first step in active compensation is a calibration measurement. This has to be performed only once for a certain set of excitation parameters (frequency and amplitude of the AC field) for a certain probe. The detection coil signal is measured for every setting of both digital potentiometers. Next, the amplitude and phase of this signal are determined using a digital phase sensitive detection (PSD) algorithm. By fitting these results, parameters ($a_0$, $a_1$, $b_0$ and $h_1$) in the following equations can be determined:

$$R_c = a_0 + a_1CA,$$

$$R_P = b_0 + b_1CP$$  \hspace{1cm} (2)

in which $R_c$ is the amplitude, $R_P$ is the phase, $CA$ is the amplitude potentiometer setting ($0...1023$), and $CP$ is the phase potentiometer setting ($0...1023$). Potentiometer settings for a desired compensation signal are given by:

$$CA = \frac{R_c - a_0}{a_1}, \quad CP = \frac{R_P - b_0}{b_1}$$  \hspace{1cm} (3)

After calibration, the excitation field is turned on and the detector signal is measured. After applying the PSD algorithm, the detector signal is given by $X_c$ and $Y_c$. The phase and amplitude of the current compensation signal ($R_c$ and $P$) are known from Eq. (2), since $CA$ and $CP$ are known. $X_c$ and $Y_c$ can be calculated:

$$X_c = R_c \cos(P_c), \quad Y_c = R_c \sin(P_c)$$  \hspace{1cm} (4)

Now, we can calculate the new compensation signal:

$$X_n = X_p - X_c, \quad Y_n = Y_p - Y_c$$  \hspace{1cm} (5)

$$R_n = \sqrt{X_n^2 + Y_n^2}, \quad \beta_p = \tan^{-1} \frac{Y_n}{X_n}$$  \hspace{1cm} (6)

Eq. (3) will be used to determine the new potentiometer settings:

$$CA = f(R_n), \quad CP = f(R_P - \pi)$$  \hspace{1cm} (7)

These settings are used in the next iteration of the DiffMag sequence.

3.3. Experimental setup

3.3.1. Device

The most important part of the device are the coils, which are shown in Fig. 3. Specifications of all coils are shown in Table 1. There are two excitations coils, one for the DC and one for the AC field. For both Litz wire is used. A transformer is connected in series to the excitation coils, but wound in opposite direction. This transformer has exactly the same mutual inductance as the excitation coils, so coupling between the coils is canceled (since the AC field would otherwise induce a current in the DC coil). Furthermore, there are two detection coils, which are in gradiometer configuration. The distance between these coils is 30 mm. Around both detection coils, compensation coils are wound.

To apply a magnetic field, a current is sent through the excitation coils. This current is provided by two power amplifiers; one for the DC coil (Servowatt DCP 390/60 50V/8A) and one for the AC coil (Servowatt VM200/48A 48V/4A). The magnetic field is verified by measuring the current that is provided by the power amplifiers. These power amplifiers are controlled by a data acquisition (DAQ) card (NI USB-6356) that is connected to a PC. All input and output signals from the DAQ card are filtered (and amplified) in a customized box with electronics to prevent aliasing. The content of this box is shown in the red rectangle in Fig. 4. The electronics box also contains two digital potentiometers to control the current sent through the compensation coils. Settings of the potentiometers are controlled by a microprocessor, which is mounted on an Arduino Uno. The signal measured by the detection coils is amplified, filtered and sent to the PC via the DAQ card. MATLAB is used both to control the system and process data.

3.3.2. Measurement protocol

All measurements were performed in a static setup. First, active compensation was performed, by iterating the process explained in Section 3.2 ten times to achieve balance. In all measurements, an excitation frequency of 2525 Hz and a sample frequency of 200 kHz were used. The length of one DiffMag sequence was set to 0.5s and 20 iterations were measured. All measurements were performed three times. Three sets of measurements were performed.

First, various amounts (25, 50, 75, 100, 250 and 500 μF Fe) of SHP-25 were measured. The samples were placed directly in front of the probe containing the detection coils and the probe was at a distance of 5 cm from the excitation coils. The currents sent were 2.4 Ampere AC and 8 Ampere DC and maximum magnetic field strengths at the location of the sample were 0.5 mT AC and 50 mT DC.

Second, the SHP-25 sample containing 500 μF Fe was measured at various distances to the excitation coils. The probe was placed at the center of the excitation coil, 1 cm above the top of the excitation coils. The sample was placed directly in front of the probe. Next, the probe and sample were moved in a straight line upwards in steps of 1 cm to a total distance of 20 cm from the excitation coils.

Last, the SHP-25 sample containing 500 μF Fe was measured in air and in proximity to a surgical steel retractor and water, in three separate measurements. The samples were placed directly in front of the detection coils and the detection coils were at a distance of 5 cm from

\[ U_{\text{det}} = \frac{dM_{\text{SPION}}}{dt} + \frac{dM_{\text{lin}}}{dt} + \frac{dH_{\text{exc}}}{dt} + \frac{dH_{\text{comp}}}{dt} \]  \hspace{1cm} (1)
the excitation coils. The retractor was placed directly on top of the excitation coils, between excitation coils and sample. Next, a square container containing 3L water was placed on top of the excitation coils, resulting in ± 4 cm water between excitation coils and sample.

**Fig. 3. Schematic representation of coils.**

**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>Wire ø [mm]</th>
<th>Inner ø [mm]</th>
<th>Outer ø [mm]</th>
<th>Turns [#]</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC excitation coil</td>
<td>2.5</td>
<td>146</td>
<td>248</td>
<td>100</td>
</tr>
<tr>
<td>AC excitation coil</td>
<td>2.5</td>
<td>252</td>
<td>266</td>
<td>20</td>
</tr>
<tr>
<td>Upper detection coil</td>
<td>0.115</td>
<td>10</td>
<td>15.5</td>
<td>720</td>
</tr>
<tr>
<td>Lower detection coil</td>
<td>0.115</td>
<td>10</td>
<td>15.5</td>
<td>-720</td>
</tr>
<tr>
<td>Upper compensation coil</td>
<td>0.115</td>
<td>15.5</td>
<td>16</td>
<td>40</td>
</tr>
<tr>
<td>Lower compensation coil</td>
<td>0.115</td>
<td>15.5</td>
<td>16</td>
<td>-36</td>
</tr>
</tbody>
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**Fig. 4. Schematic representation of signal filtering and amplification. All components in the red rectangle are present in a customized electronics box.**

**Fig. 5. Calibration results showing amplitude and phase of the signal from the detection coils for every setting of the potentiometers. At the highest values of CA the amplitude bends, because the DAQ card has a range of ± 10 V.**
4. Results

4.1. Active compensation

Fig. 5 shows calibration results. The amplitude and phase of the signal from the detection coils is shown for every setting of the potentiometers.

Fig. 6 shows ten iterations of active compensation. At the start, the excitation field is disturbing the signal, but it is gradually canceled out. After ten iterations, the probe is balanced and SPIONs can be measured.

4.2. SPION measurements

Static particle measurements are shown in Fig. 7. SHP-25 can be measured down to 25μg Fe.

Measurements at various distances to the excitation coils for the SHP-25 sample containing 500μg Fe are shown in Fig. 8. Measurements are possible up to 20 cm from the top of the excitation coils. Error bars show ± one standard deviation.

Fig. 8. DiffMag counts at various distances to the excitation coils. An SHP-25 sample containing 500μg Fe was placed directly in front of the detection coils. The detection coils and sample were moved away from the excitation coils in steps of 1 cm. Error bars show ± one standard deviation.

SHP-25 sample containing 500μg Fe are shown in Fig. 8. Measurements are possible up to 20 cm from the top of the excitation coils.

Fig. 9 shows DiffMag and AC magnetometry measurements on the SHP-25 sample containing 500μg Fe. The sample was measured in air, and in proximity to a surgical steel retractor and water. On the contrary, AC magnetometry counts are increased in presence of a surgical steel retractor, and decreased in presence of water. Furthermore, the standard deviation is much larger in AC magnetometry measurements compared to DiffMag.

5. Discussion

Our novel laparoscopic probe for in vivo detection of SPIONs has five main advantages. First, it makes use of small field strengths. As a result, energy consumption is limited and handheld detection becomes possible. Second, separation of excitation and detection coils makes it
5.1. Performance in relation to clinical needs

Currently, the minimum amount of SPIONs that can be identified with our novel probe contains 25 μg Fe. In the clinical situation, a sentinel node contains 60–220 μg Fe [12]. This means that our probe is already sensitive enough to detect sentinel nodes. However, this detection limit of 25 μg Fe was determined for measurements where SPIONs were placed directly in front of the detection coils. Biot-Savart law is used to predict the maximum detection depth of a sentinel node. Fig. 7 shows a linear relation between DiffMag counts and amount of iron in the sample. This linear relation is used to calculate the counts induced by a typical sentinel node. The empty coil measurement shown in Fig. 7 provides the threshold, or minimum number of detectable counts. The depth sensitivity of a sentinel node containing 60–220 μg Fe is currently 14–24 mm. Reducing noise in the system, as described in Section 5.2, will improve sensitivity of the probe and consequently the maximum detection depth.

For example, in breast cancer patients, sentinel nodes have a mean depth of 4 cm [11]. However, in laparoscopic surgery sentinel nodes are not measured through the skin, but the probe is placed directly on the fatty tissue containing the lymph nodes [23]. To conclude, the present sensitivity of our probe is already clinically usable.

5.2. Improvements before clinical implementation

Although sensitivity of the probe is already clinically usable, the probe can be improved for clinical use in four ways. First, it is essential that movement of detection coils is possible during SPION measurements. This can be achieved by implementation of active compensation in the DiffMag protocol. The signal of one block of the DiffMag sequence will be used to calculate new compensation values and thus to balance the probe. The length of a DiffMag sequence needs to be reduced to enable compensation in real time and faster movement of the probe.

Second, the diameter of the probe must be reduced. For clinical usage it must fit through a standard 12 mm trocar.

Third, sensitivity of the probe can be improved. This will lead to measurement of either a lower quantity of SPIONs, or measuring a sample at a larger distance from the detection coils (measuring nodes that are located deeper in tissue). Currently, there are distortions on the measurement signal. Part of these distortions are caused by the 50 Hz harmonics. This is why we now measure at 2525 Hz instead of 2500 Hz. Furthermore, the power amplifiers seem to introduce noise. We also want to amplify the probe signal directly after the detection coils instead of in the electronics box, to avoid signal loss when the signal is transferred through a cable. Improving these electronics in our setup will improve sensitivity of the probe.

Finally, it would help to make the excitation field more homogeneous. This would make balancing of the probe much easier. If we can achieve a perfectly homogeneous field, the excitation field is the same at every location of the probe. As a result, the field is equal in both detection coils. The coils will be passively balanced, making active compensation less crucial. Another advantage of a homogeneous excitation field is that DiffMag counts are in that case not dependent on the location of the sentinel nodes. Fig. 8 shows that DiffMag counts decrease when distance to the excitation coils is increased. However, achieving a sufficiently large homogeneous excitation region would require a more complicated setup with large coils, making a surgical procedure more difficult.

It is hard to say how the improvements described in this section will affect the measurements. The theoretical noise limit is the resistance of the detection coils. We are already close to clinical needs, so slight improvements will make this probe usable in the clinic.

6. Conclusion

A novel probe for in vivo detection of SPIONs has been developed. A unique feature of this probe is mechanical separation of excitation and detection coils. Active compensation was developed and demonstrated, allowing independent movement of the detection coils with respect to the excitation coils. With our current hardware it is possible to measure as little as 25 μg of SPIONs. Furthermore, measurements are successful at various distances from the excitation coils, showing the possibility to move the detection coils. Measurements are successful because we use DiffMag. Distortions in balance of the probe do not influence DiffMag...
measurements. Finally, both surgical steel and diamagnetism of tissue have minor influence on DiffMag measurements. In conclusion, this paper shows promising first steps towards laparoscopic sentinel node biopsies, since it enables identification of magnetically marked nodes in the diamagnetic human body.

Acknowledgements

Financial support from the Netherlands Organization for Scientific Research (NWO), under the research program Magnetic Sensing for Laparoscopic (MagLap) with project number 14322 is gratefully acknowledged. Furthermore, the authors would like to thank A. Vugelers for creating Fig. 1, S. Draack for help with creating Fig. 3, E.R. Nieuwenhuis, A.M. Hoving and M.E. Kamphuis for proofreading and L. Molenar for discussing content.

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