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# Ultrasensitive detection of bioanalytes based on signal amplification of coil-integrated giant magnetoimpedance biosystems

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ABSTRACT

This article reports a novel separable bioanalyte detection system that combines magnetic nanoparticle labels with giant magnetoimpedance (GMI). The GMI sensor was fabricated via standard microfabrication techniques, and a micro-solenoid coil was integrated with the sensing elements to amplify the signal tags. To verify the feasibility of this strategy, streptavidin-coupled Dynabeads and a-fetoprotein (AFP) bioconjugates were utilized as model analytes, while double antibody sandwich immunoassays and streptavidin-biotin binding assays were employed to immobilize and label AFP on an Au-film coated wafer. The resulting biosystem exhibited markedly improved sensitivity compared to other similar biosensors: A minimum detectable limit of 3 ng/ml for streptavidin-coupled Dynabeads and 0.2 ng/ml for AFP were achieved, respectively. In addition to its very high detection sensitivity, the proposed biosystem can be conveniently manipulated, is not contaminated or damaged by chemical solutions, and is reusable without cleaning. The results presented here may considerably enhance the practical applications of GMI sensors in bio-magnetic-field sensing.



## 1. Introduction

The giant magnetoimpedance (GMI) effect has become a popular research object in the magnetic materials field, due to its potential for the development of high-performance magnetic sensors [1,2]. The GMI effect is characterized by a large change in alternating current (AC) impedance upon the application of a static external magnetic field [3]. In soft magnetic thin films, the GMI effect is caused by the skin effect [4,5] as a consequence of variations in penetration depth, induced by the applied static magnetic field via modification of the transverse permeability [3,6]. This effect has been extensively studied during recent decades in soft amorphous ferro-magnetic wires [7], thin films [8], and ribbons [9] for the development of GMI-based sensors.

Magnetic beads (MBs) have attracted considerable research interest due to their wide applications for magnetic labeling and separation [10,11]. As magnetic labels of molecules, MBs offer unique advantages such as minimal background signals, physico-chemical stability, and biocompatibility, thus enabling detection and magnetic manipulation, both in vitro and in vivo, without affecting biological interactions in the host material. Streptavidin-coupled Dynabeads are uniform polymer spherical magnetic beads that have been made magnetizable and superparamagnetic. They define the surface for adsorption or coupling of various bioreactive molecules and cells and have become the gold standard for both isolation and handling of biotinylated nucleic acids [12], antibodies, and other biotinylated ligands [13–15]. The stray field of Dynabeads induced by an applied magnetic field can furthermore be used to detect and quantify drug molecules or biomarkers. In short, the detection of streptavidin-coupled Dynabeads plays a central role in many biomedical applications.

Recently, double antibody sandwich immunoassays and streptavidin-biotin binding assays based on giant magnetoimpedance sensors have been proposed for Dynabead-labeled biomarker detection [16]. However, the existing bio-detection methods based on GMI sensors suffer from several drawbacks: 1) the efficient working magnetic field-range of recently reported GMI-based detection methods is limited to ~20 Oe. This means that the applied magnetic field available for biotesting will also be in this range. Fonnum et al. [17] reported that the magnetic field needed to force magnetic beads into saturation state lies above 100 Oe. Hung et al. [18] also reported the saturation magnetic field needed for streptavidin-coupled Dynabeads to be ~40 Oe. This means that the nano-particles during a given GMI test are frequently unsaturated

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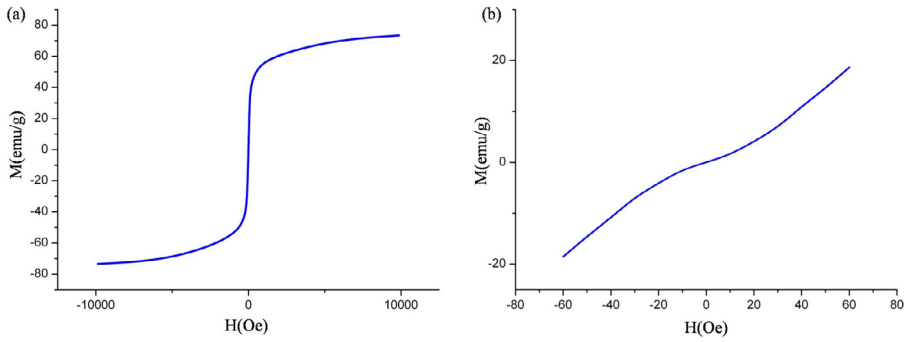


**Fig. 1.** Magnetic susceptibility of streptavidin-coupled Dynabeads: (a) full view, (b) partial enlargement for a field range of −60 to 60 Oe.

or in a very low-level magnetized state. In a previous study, Wang et al. [16] have reported that a low-level magnetic field leads to small magnetization of Dynabeads, i.e., a reduction in sensitivity of the biosystem as a whole. 2) Due to the low-level magnetization of Dynabeads, an in situ detection method had to be established [16], where the bio-analytes are directly attached on the surface of the sensor via a series of complex biochemical reactions that increase the reciprocal influence between the sensors and the Dynabeads, thus improving sensor sensitivity. Even in the presence of a protective layer such as aluminum dioxide ($Al_2O_3$) [16], the sensor is prone to contamination and damage, while the surface biomarker layer is prepared. Furthermore, the presence of a very thick protective layer increases the distance between Dynabeads and the GMI sensor, rendering the primary purpose of improving sensor sensitivity redundant. Moreover, the sensor must be repeatedly washed for reuse, making continuous quantitative measurement rather challenging, while modifying the GMI sensor's surface can actually reduce its GMI performance and stability.

Fig. 1 shows the magnetic susceptibility curve of streptavidin-coupled Dynabeads (Invitrogen Co. Ltd); the beads are clearly very sensitive to the magnetic field. As the applied field increased by 10 Oe, the magnetization of Dynabeads increased nearly 100% in a field range of 20–50 Oe. This observation suggests that improving the magnetization of Dynabeads may be an effective approach to enhancing the detection sensitivity of magnetosensor-based biosystems. Very few researchers have explored this approach; however, studies to this effect on GMI-based biosystems are virtually nonexistent.

Detecting magnetism in biomarker-labeled Dynabeads has considerable application potential with regards to the early identification of several diseases. The bioanalyte alpha-fetoprotein (AFP) is an important plasma protein, mainly produced by the yolk sac and the liver during fetal development. Recent research has revealed that AFP is directly involved in hepatocellular carcinoma, and is the most reliable predictor of primary liver cancer [19]. In fact, an elevated AFP level is a factor in the diagnosis of several diseases [20,21]. Accordingly, high-sensitivity AFP measurement may provide valuable information for both medical diagnosis and treatment opportunities.

At present, commercially available quantitative immunoassay methods, such as immune-turbidimetry and chemiluminescence, are based on enzyme-linked immuno sorbent assay-tests [22,23]. These methods tend to be either time-consuming or entirely lab-based and therefore non-transferrable to point-of-care platforms. Combining a magnetoimpedance immunosensor with a sandwich immunoassay allows for quick AFP measurement; however, the in situ detection method used in previous studies may not be suited for continuous and quantitative identification [16]. There is urgent demand for next-generation bioanalytical systems with high sensitivity and rapid response for the quantitative determination of AFP.

Considering the limitations of recent GMI-based biosensing methods, we conducted this study, targeted to explore an innovative signal amplification strategy based on a composite-structure magnetoimpedance sensor. As a departure from previous studies on this subject, we employed a coil-integrated GMI biosensor to improve the magnetization level of magnetic particles and a separable detection method for Dynabeads and biomarkers. The influence of the coil-integrated composite structure on bioassay performance was investigated in detail; various concentrations of streptavidin-coupled Dynabeads were quantified to ultimately achieve a detection concentration as low as 3 ng/ml (30 particles), i.e., a biosystem with extremely high sensitivity was effectively established. AFP biomarker detection was carried out in this biosystem to verify the biomarker-detection performance of the system. A minimum detectable AFP concentration of 0.2 ng/ml was obtained with a biosystem response below 5 s. Since the GMI sensor can be easily fabricated via the micro electromechanical systems (MEMS) technique and is compatible with lab-on-chip technology, the entire biosensing system can be miniaturized into an integrated biochip for real-time detection of biomolecules.

## 2. Experimental Procedure

### 2.1. Materials and methods

The Supplementary Information section provides experimental details including the reagents we used, the device fabrication process, and the sample preparation process. It is worth mentioning that the bio-sample we utilized in this study was prepared on a separate glass substrate from the sensor substrate to prevent the sensor from being contaminated by chemical solutions. The proposed GMI biosensor is composed of symmetrical meandering $Ni_{77}Fe_{23}/Cu/Ni_{77}Fe_{23}$ sensing elements for biomagnetic measurement and with an integrated-3D-solenoid coil for signal amplification. The double $Ni_{77}Fe_{23}$ layers were mainly responsible for the superimposed GMI effect, while the Cu layer is the pathway for the AC current. The 3D coil can be subdivided into three sections during fabrication: The bottom segment, the top segment, and the vias. Vias were used to connect two other parts, which were fabricated on separate layers. Images of the fabricated GMI sensor are provided in Fig. 2.

### 2.2. GMI-based measurement method for Dynabead label detection

During the test, a HP 4194A impedance-meter was connected to the sensing elements to provide a high-frequency AC and to measure the impedance of the sensing elements. A static external magnetic field ($H_e$) was applied along the longitudinal direction of the sensing elements to induce the desired GMI effect. The