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Single magnetic microsphere placement and detection on-chip using current line designs with integrated spin valve sensors: Biotechnological applications

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Superparamagnetic labels, 400 nm dextran iron oxide particles and 2 μm polymer encapsulated iron oxide microspheres, with biomolecules immobilized on the surface, e.g., the enzyme horseradish peroxidase (20–40 molecules per label) were controllably placed on chip sites (5×15 μm^2) using tapered Al current lines (10–20 mA current) and moved to and from adjacent spin valve sensors [2×6 $\mu m,^2$, magnetoresistance (MR) $\sim$5%]. Average MR signals of 1.2 and 0.6 mV were obtained for the detection of bulk numbers of 400 nm and 2 μm labels respectively using an on-chip field of 15 Oe and a sense current of 5 mA. The moment per label was calculated at 5×10^{-13} emu for the 400 nm labels and 5×10^{-12} emu for the 2 μm labels, illustrating the higher density of the 400 nm particles. MR signals of $\sim$100 μV were obtained for single 2 μm labels positioned over the spin valve sensor using an on-chip field of 15 Oe and 8 mA sense current. The corresponding sensor saturation occurred at $\sim$1 mV, with a noise level of $\sim$10 μV. The estimated maximum MR signal for one 2 μm label directly on top of the sensor was $\sim$400 μV. Biotechnological applications include high sensitivity biosensors and biochips for protein and DNA screening. © *2002 American Institute of Physics.* [DOI: 10.1063/1.1451898]

INTRODUCTION

The discovery of antiferromagnetic interlayer exchange coupling[1] and the giant magnetoresistive (MR) effect[2] in the mid 1980s opened up a number of applications for magnetic nanostructures. These include magnetic recording media, magnetoresistive sensors, read heads, and magnetic random access memory. In the last few years magnetoresistive sensors have also been proposed as potential detection components in biological devices such as high sensitivity biosensors and biochips based on a magnetic labeling platform technology.[3,4] In this article we present two advances in the development of such devices. First, the construction of on-chip current line structures to precisely control the movement of magnetically labeled biomolecules and second, the use of spin valve sensors to detect the magnetic labels. Two types of magnetically labeled biomolecules were employed. A widely used analytical enzyme, horseradish peroxidase[5] (HRP, E.C. 1.11.1.7), and the protein streptavidin.

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MATERIALS AND METHODS

Two types of magnetic labels were used. A developmental sample of 400 nm dextran–iron oxide (70%) particles (Nanomag®-D, Micromod, Germany, stock concentration 83 mg/ml) and 2 μm polymer encapsulated iron oxide (15%) microspheres (Micromer®-M, Micromod, stock concentration 25 mg/ml). These particles and microspheres (Table I), which we will refer to as labels, are available with a variety of surface functionalization for the attachment of biomolecules. HRP was immobilized to Nanomag®-D functionalized with free carboxyl groups using the negatively charged polymer polyethylenimine, the crosslinking reagent glutaraldehyde, and para-benzoquinone. HRP was also immobilized to Micromer®-M with free amino functionality using the common glutaraldehyde methodology. The number of active immobilized HRP molecules per label was calculated via the determination of enzyme activity using the guaiacol reaction in an ultraviolet/visible spectroscopic assay. This provides an equivalent quantity of active HRP per particle via comparison of the activity of label suspensions with known HRP concentrations. This is then converted to molecular numbers using Avogadro's number. Micromer®-M samples with pre-

TABLE I. Properties of Nanomag®-D and Micromer®-M superparamagnetic labels.

400 nm particles (Nanomag®-D)	2 μm microspheres (Micromer®-M)
Bell-shaped size distribution: 200–600 nm	very uniform size and shape: spherical
70%–80% magnetite+maghemite crosslinked with dextran	microconed magnetite 15% in copolymer composite
stock: 83 mg/ml particles	stock: 25 mg/ml microspheres
density: 4.0 g/ccm	density: 1.4 g/ccm
saturation magnetization: >60 emu/g ($H>10\,000$ Oe)	saturation magnetization: >34 emu/g ($H>10\,000$ Oe)

immobilized streptavidin were obtained directly from Micromod. The magnetic moment of both types of label was determined by drying 40 μl aliquots of known stock label suspensions (25 or 83 mg/ml) in a vacuum oven for 100 °C for 1 h, measuring the dry weight of the samples, and then measuring the magnetic susceptibility with a vibrating sample magnetometer. The moment per label was calculated using the supplier's data on the weight, density, and number of labels per unit weight for each sample. For the 2 μm particles $\chi\sim 0.06$ emu/(g Oe) and for the 400 nm particles $\chi\sim 0.25$ emu/(g Oe).

Spin valve sensors with dimensions 2 μm$\times$6 μm were fabricated with the structure Si/Ta20 Å/NiFe30 Å/CoFe20 Å/Cu28 Å/CoFe25 Å/MnIr60 Å/Ta25 Å with an average MR of 5%. Six sensors per chip were integrated within the final current line structure, each sensor having two associated current lines. The width of sensor line connections was stepped down to 5 μm at sensor sites from 50 μm at the sensor pads, while current lines were tapered from 150 to 5 μm at sections adjacent to the sensors [Figs. 1(a)–1(c)]. The chip surface was passivated with a layer of silicon dioxide (3000 Å). Approximately 50 (8$\times$8 mm) chips were fabricated per 3 in. silicon wafer and cut using a dicing saw. Individual chips were then mounted on 40 pin chip carriers and the electrical connections made via wire bonding of the contact pads. Wire bonded areas were protected using a silicon gel. The mounted chip was then connected to a multimeter and three power sources: one for the sensor line and two for its associated current lines. Magnetic label movements' on chip and corresponding sensor resistance changes were observed simultaneously by positioning of the chip carrier on the stage of a light microscope. All movement and detection experiments were performed in small volumes (5–10 μl) of water or phosphate buffer (100 mM, *p*H7) containing low label concentrations, which were applied to the chip using an autopipette. The spin-valve transfer curve was linearized with its sense current (5–8 mA). An in-plane magnetic field, perpendicular to the length of the sensor, created by a horseshoe magnet current (15–20 Oe) was also used to induce a measurable moment within the superparamagnetic labels. Control experiments were performed using fluid samples without magnetic labels under identical conditions to determine any potential background signal contributions. These included the addition of fluid and other environmental temperature changes, electrical interference, and changes of current through the aluminum lines associated with each sensor.

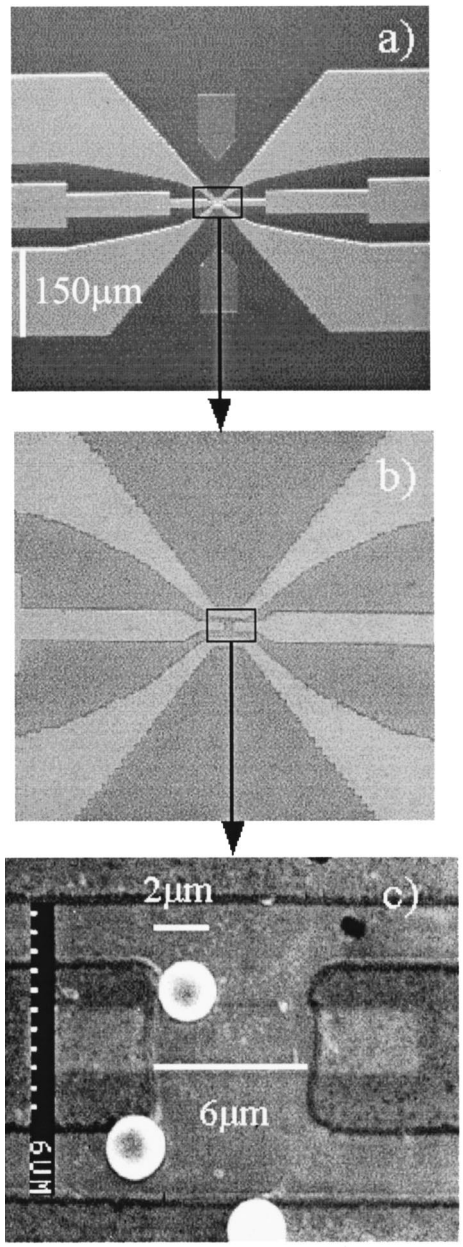

FIG. 1. Light microscope (a), (b) and SEM (c) images of current line, sensor line, and sensor integration and dimensions. (c) Shows three 2 μm Micromer®-M microspheres: one touching the sensor, one touching the sensor line and a third on the bottom current line.

Noise was reduced using coaxial cables and housing of the connection board in a homemade noise reduction box.

RESULTS AND DISCUSSION

In preliminary experiments to demonstrate the use of current line structures to control the movement of magnetic labels, a simple aluminum tapered line was constructed, starting with 100 μm width and with a central section of a narrower width (10 μm). This was then immersed in fluid containing 400 nm Nanomag®-D labels and a current of 10 mA was passed through the line. The labels were seen to move to the line while current was passing and to move away from the line after the current was switched off. Furthermore, the labels tended to move more readily to the nar-

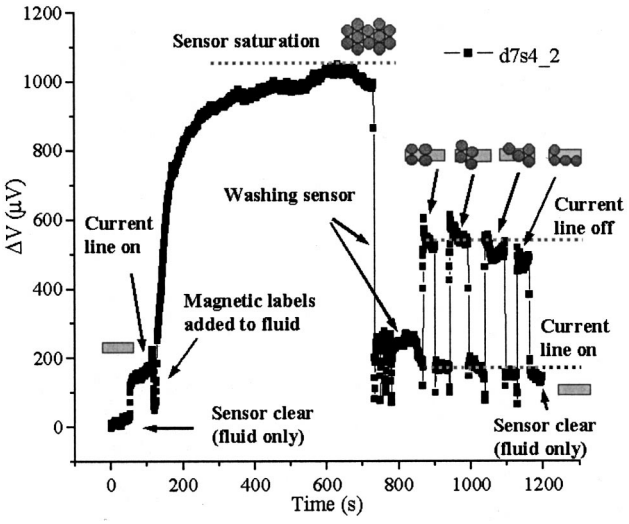

FIG. 2. Real-time data for the movement and detection of Micromer®-M labels with immobilized horseradish peroxidase.

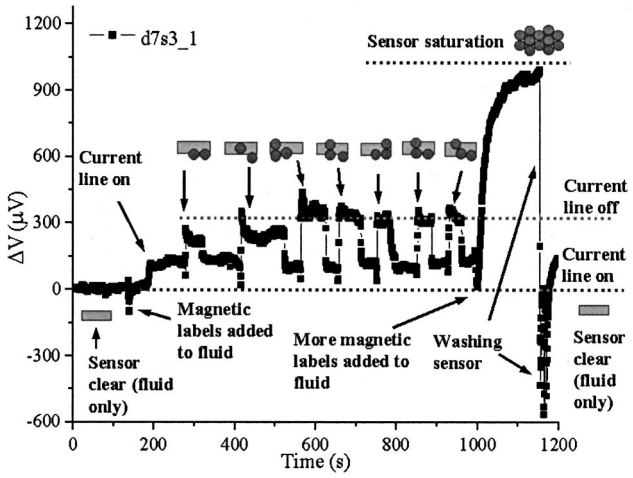

FIG. 3. Real-time data for the movement and detection of Micromer®-M labels with immobilized streptavidin.

rower section of the line, where the local magnetic field generated was higher. This principle was then used to design various on-chip current line structures, which evolved into the structure shown in Fig. 1. Currents of 10–20 mA were passed through the tapering current lines to focus labels at sites ($5\times15\ \mu m^2$) adjacent to spin valves. The labels were then moved to and from the sensor by switching the current line off and on again as required.

Both 400 nm and 2 μm labels could be detected in bulk numbers ($\gg10$) using this approach. The initial average MR signals at 5 mA sense current were 1.2 mV (0.3%) and 0.6 mV (0.15%), respectively. The observed signals for Nanomag®-D were higher due to the smaller size and hence higher density of labels that can accumulate on the sensor surface. The moment values per unit weight were ~3.7 emu/g of 400 nm labels and 0.9 emu/g of 2 μm labels, while the relative moment values for single labels calculated at 15 Oe were 5×10^{-13} emu/400 nm label and 5×10^{-12} emu/2 μm label. An immediate disadvantage of the use of 400 nm Nanomag®-D labels was their tendency to cluster readily in an external field, preventing the determination of single label signals. However, Micromer®-M microspheres could be controlled and detected as single labels. Subsequently, all additional detection experiments were performed using Micromer®-M labels. The real time detection signals for Micromer®-M with immobilized HRP molecules can be seen in Fig. 2. Spectroscopic assays on the activity of these labels indicated ~20–40 active molecules of HRP per label, depending on the particular conditions of the immobilization procedure. Figure 3 shows an equivalent chart for the movement and detection of Micromer®-M labels with immobilized streptavidin. These plots and additional data were used to calculate average detection signals for 1–6 labels and saturation signals for different sensors (>6 labels). Single labels gave a response on the order of 100 μV with sensor saturation occurring around 1 mV. The noise level was typically 10 μV (1% saturation). These signals were found to be reproducible with respect to sensor and chip. As a means of comparison we performed theoretical calculations for the signal based on the magnetic moment of a single label positioned directly (1.3 μm) above the sensor. This resultant value was ~400 μV, but this represents a maximum signal and assumes that there is no fluid between the label and the sensor surface.

CONCLUSIONS

The present work provides a means to move and detect nanometer or micrometer sized magnetic labels and we have successfully demonstrated the detection of single 2 μm magnetic microspheres with a small number of active biomolecules attached. Our present goal is to use this system to demonstrate the detection of the binding of streptavidin functionalized magnetic labels to sensor bound biotin,[6] which will demonstrate the use of this system to detect biomolecular recognition and provide a vehicle for the placement, detection, and study of other biomolecules and other biomolecular interactions, in particular single DNA molecule interactions.[7] Consequently we foresee a wide range of biotechnological applications for this system, including miniaturized high sensitivity biosensors and biochip devices.

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