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Spin valve sensors for ultrasensitive detection of superparamagnetic nanoparticles for biological applications

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Abstract

We present giant magnetoresistance (GMR) spin valve sensors designed for detection of superparamagnetic nanoparticles as potential biomolecular labels in magnetic biodetection technology. We discuss the sensor design and experimentally demonstrate that as few as ~23 monodisperse 16-nm superparamagnetic Fe₃O₄ nanoparticles can be detected by submicron spin valve sensors at room temperature without resorting to lock-in detection. A patterned self-assembly method of nanoparticles, based on a polymer-mediated process and fine lithography, is developed for the detection. It is found that sensor signal increases linearly with the number of nanoparticles.

Keywords

Spin valve sensor; GMR; Magnetic nanoparticle; Biodetection; Biosensor

1. Introduction

For the past several years, giant magnetoresistance (GMR)-based magnetic biodetection technology, which involves labeling biomolecules with magnetic micro- or nanometer-sized particles and detecting the magnetic fringing fields of the particle labels by GMR sensors after capture by target-probe biomolecular recognition, has received increasing research and development efforts [1–14]. This is because the GMR biosensors are promising for sensitive, large-scale, inexpensive, and portable biomolecular identification. They are also compatible with standard silicon IC technology, and suitable for integration into a lab-on-chip system. Compared to the superconducting quantum interference device (SQUID)-based ultrasensitive magnetic biodetection [15,16], the GMR technology has advantages of room-temperature operation, less complex instruments, and hence more portable and flexible implementation. Moreover, given the nature of the solid-state thin film sensors and the IC compatible fabrication, the GMR biosensors can be integrated into a very high density, individually addressable array similar to the magnetic random access memory (MRAM), and hence such a GMR sensor array will be well suited for multi-analyte biodetection [2,3].

Up to date, most magnetic particle labels in the literature are micron or submicron sized, usually composed of a polymer matrix with imbedded magnetic nanoparticles or a polymer core coated with magnetic nanoparticles [1–10]. However, in order to achieve ultrahigh biodetection

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sensitivity, e.g., single molecule detection, the dimension of magnetic particle labels should be comparable to that of biomolecules. In the case of detecting DNA fragments, it is ideal to have the particle labels at 20 nm or smaller in diameter [17]. Such small nanoparticles would not block bimolecular interactions, such as hybridization between complementary gene fragments. Moreover, one nanoparticle label may be conjugated with one or at most a few DNA fragments, which will help establish a quantitative relationship with a sufficient accuracy between the number of captured particle labels and the actual biorecognition events. In contrast, it is quite difficult to do so with microbeads because of their large size mismatch with biomolecules. The monodispersity of crystalline magnetic nanoparticles in both size and magnetic moment [18,19] also benefits the signal quantification, in contrast to the large variations of the microbeads in magnetic moment [1,10].

Given all these desirable properties, magnetic nanoparticles with a diameter of 20 nm or smaller become desirable biomolecular labels for ultrasensitive, highly quantitative magnetic biodetection technology. On the other hand, such tiny magnetic nanoparticles are a great challenge to the detectors, because their magnetic moments are very low due to their limited physical volume, relatively large surface area, and significant thermal disturbance to magnetic moments, i.e., superparamagnetism. In this paper, we present a GMR spin valve sensor designed and fabricated for detecting monodisperse superparamagnetic nanoparticles as potential biomolecular labels. The sensor design will be discussed first, and then we will present experimental results demonstrating the quantitative detection of a few tens to hundreds of magnetic nanoparticles (16-nm Fe₃O₄) by spin valve sensors at room temperature.

2. Sensor design

2.1. Spin valve sensor

The physical volume of magnetic nanoparticles limits their magnetic moments and hence their magnetic fringing fields. Therefore, the nanoparticle sensor is required to possess high field sensitivity. For that reason, a GMR spin valve has been chosen as the sense element for magnetic nanoparticle detection because of its high sensitivity to low magnetic fields [20]. The spin valve with a synthetic antiferromagnet pinned layer is the preferred structure for its large dynamic range of field, good thermal stability, and design flexibility [21], and is actually used to fabricate the spin valve sensors presented in this paper.

Fig. 1 shows schematic drawings of a spin valve sensor designed for detecting magnetic nanoparticles that are conceptually immobilized onto the sensor surface via DNA hybridization. Unlike the binary digital output of a spin valve cell in nonvolatile memory applications [22], a linear analog signal is desired from the spin valve sensors for quantitative biodetection. For this purpose, the spin valve sensor is best configured in an orthogonal magnetization state [23]. Fig. 1(a) shows a patterned rectangular spin valve sensor with its pinned magnetization M_p fixed in the transverse or y direction and the free magnetization M_f rotating freely in the sensor plane. In this configuration, the sensor resistance can be expressed as $R = R_0 + (1/2)\Delta R_{\max} \sin \theta_f$, where $\Delta R_{\max} = R_{AP} - R_P$ is the sensor resistance change between the antiparallel (R_{AP}) and parallel (R_P) magnetization configurations, $R_0 = R_P \Delta R_{\max}/2$ is the sensor resistance at the orthogonal configuration, and θ_f is the orientation angle of free layer magnetization with respect to the longitudinal or x direction. For an external field applied in the transverse direction within a certain range, $\sin \theta_f$ is linear or approximately linear with the field, leading to a linear dependence of sensor resistance on the field too.

For our spin valve sensors, a couple of methods have been used to establish such an orthogonal magnetization configuration. We deposited the free and pinned layers in different magnetic fields that were orthogonal to each other. Moreover, as shown in Fig. 1(a), the sensors were patterned in such a rectangular shape that the pinned magnetization is aligned in the transverse

direction and the shape anisotropy will then align the free layer magnetization in the longitudinal direction. In this magnetic configuration, the transverse direction is the field-sensitive direction of the spin valve sensors.

To avoid unwanted Barkhausen noise in the signal output of a spin valve sensor, we need to magnetically stabilize the sensing free layer. As used in magnetic recording heads, a permanent magnet bias would be effective for free layer stabilization for the spin valve biosensor, but with a modest increase in fabrication complexity. For our prototype spin valve sensors, especially those with a submicron-scale width (w), we utilize shape anisotropy to stabilize the free layer. In addition, an external bias field H_b , as shown in Fig. 1(a), can also be applied to enhance the free layer stabilization. Moreover, we overlay thick aluminum leads on the end segments of the spin valve stripe where complex domain structure is most likely to form, as shown in Fig. 1. This lead configuration will shunt the electrical sense current (I_{sense}) away from those regions and hence reduce possible Barkhausen noise.

Defined by the aluminum leads, the central segment of a spin valve sensor generates the electrical signal when a sense current passes through it, and therefore is called the “electrically active area”. When designing the electrically active area, we should consider the sensor surface area available for biochemical reactions, which is called the “biologically active area”, the sensor signal strength, and noise. As illustrated in Fig. 1, the biologically active area is an area of the surface located inside the electrically active area and where biorecognition, such as DNA hybridization, and nanoparticle immobilization occurs. The biologically active area can be a physically separate layer, such as gold, deposited on the sensor to suit the surface chemistry requirements for biochemical reactions. In principle, the biologically active area can be as large as the whole electrically active area to maximize the available biochemical reaction sites. However, for good signal quantification, the biologically active area should rather be confined to the central portion of the electrically active area and have a high aspect ratio, because the spatial uniformity of the sensor signal degrades significantly when magnetic nanoparticles are located close to the sensor edges [12]. Nonetheless, given a sensor width, the longer the electrically active length is, the larger the biologically active area can be.

A long electrically active length is also beneficial to the sensor signal, because the longer the electrically active length is, the more magnetic flux from magnetic nanoparticles the electrically active area collects, and therefore the larger the sensor signal is due to the nanoparticles. On the other hand, the sensor noise increases with the electrically active length, because electrical noises, such as $1/f$ noise and Johnson noise increase with the sensor resistance which is proportional to the electrically active length. Therefore, the electrically active length should be a result of balancing all these factors. Similar trade-off exists to the sensor width. It is straightforward to see that the narrower the sensor width is, the more sensitive the sensor is to the magnetic fields of the nanoparticles. However, narrowing sensor width will increase sensor resistance and hence noise, and also decrease the biologically active area.

2.2. Detection methods

The basic idea of detecting magnetic nanoparticle labels is to excite the superparamagnetic nanoparticles with a magnetic field and then to detect their magnetic responses (moments or fields) by the GMR sensors [1–13]. The magnetic excitation field, however, can be applied in different forms and directions. For example, a DC or AC magnetic excitation field can be applied, and this excitation field can be either in the sensor plane or perpendicular to the sensor plane, leading to four detection modes: in-plane DC [4,6,8,13], in-plane AC [7], vertical DC [5,10], and vertical AC modes [1–3,12], as illustrated in Fig. 2 where the excitation field is denoted as H_t . In addition to the excitation field, a constant DC bias field H_b may also be applied in the sensor plane for different purposes in different detection modes. In this paper, we define

the sensor's response to the excitation field H_t as the sensor signal. The static DC bias field H_b only affects the base state of the spin valve sensor.

As shown in Fig. 2(a and c), in the in-plane detection modes, the magnetic excitation field H_t is applied in the transverse direction, which is the field-sensitive direction of the spin valve sensor, and the DC magnetic bias field H_b , if applied, is in the longitudinal direction. The DC bias field H_b has three roles in the in-plane modes: (1) stabilizing the free layer in the spin valve sensor, (2) defining a proper working point for the sensor, and (3) providing an initial polarization or magnetization for nanoparticles. However, for the in-plane detection modes, the bias field is not really necessary if other free layer stabilization methods, such as permanent magnet bias or a large shape anisotropy, are available. In fact, the application of H_b in the longitudinal direction will decrease the field sensitivity of the spin valve sensor in the transverse direction, because H_b will increase the magnetic stiffness of the free layer magnetization. Moreover, the magnetic nanoparticles do not need an initial polarization in the in-plane modes, because the excitation field H_t is already in the transverse direction and the induced effective magnetic fields from the nanoparticles will also be exerted in the same direction on the sensor. Based on the above reasons, the DC bias field may be needed only for a micron-wide spin valve sensor, mainly to stabilize its free layer, whereas for a submicron-wide sensor it may not be needed if the free layer is already in a single domain state.

For the vertical modes, the excitation field H_t is applied in the vertical direction. The DC bias field, however, needs to be applied in the transverse direction for a spin valve sensor [12], as shown in Fig. 2(b and d). In contrast to GMR multilayer sensors whose resistance is affected only by the field magnitude [1–3,5,10], a spin valve sensor is sensitive to the field direction as well. If no DC bias field is applied, the magnetic fringing field of a vertically magnetized nanoparticle will be radially symmetrical in the sensor plane [24], leading to a zero net field. This is not a problem for a GMR multilayer sensor, but it is an important issue for a spin valve sensor because the resistance of a spin valve sensor under such a radially symmetrical field will not change. However, we know that the magnetic susceptibility (χ) of superparamagnetic nanoparticles is not constant but a function of applied field H , i.e., $\chi(H) = M/H = m_s L(\alpha) / HV_p$, where the Langevin function $L(\alpha) = \coth \alpha - 1/\alpha$, $\alpha = m_s H/kT$, m_s is the saturation moment of a nanoparticle, V_p the nanoparticle's volume, k the Boltzmann's constant, and T is the temperature. Therefore, by applying a transverse bias field H_b in the vertical detection modes,

we can not only gain a nonzero transverse magnetic moment $m_y = \chi \left(H = \sqrt{H_b^2 + H_t^2} \right) H_b V_p$ for the nanoparticle, but also make this transverse moment vary with the vertical excitation field H_t via the field-dependent magnetic susceptibility χ . Hence, due to this transverse moment, the average in-plane field from the nanoparticle will also vary with H_t . As a result, the resistance of the spin valve sensor with captured nanoparticles will change in response to the vertical excitation field. For the above reasons, a transverse DC bias field is indispensable in the vertical detection modes for a spin valve sensor.

One advantage of the vertical modes over the in-plane modes is that there is no net signal output when no magnetic nanoparticles are present, because a spin valve sensor is insensitive to a vertical excitation field. On the other hand, the in-plane modes have a higher tolerance to the possible misorientation of the excitation field H_t than the vertical modes due to the same reason. A slight tilting out of the sensor plane of H_t would not affect the sensor signal much because of the insensitivity of the spin valve to the vertical field. In the vertical modes, however, a slight tilting of H_t away from the normal direction will introduce an in-plane component of H_t , which would, in turn, change the zero-particle signal from null to a finite value that might be comparable to the true signal from the nanoparticles.

As mentioned before, the magnetic excitation field H_t can be DC or AC. Compared to a DC excitation, an AC excitation will generate an AC MR signal from a spin valve sensor, which therefore can be measured with a lock-in amplifier to achieve a high signal-to-noise ratio (SNR) [1–3,7,9,12]. The in-plane AC mode signal has the same frequency ($1f$) as the AC excitation field H_t [7], whereas the vertical AC mode signal is at twice the frequency ($2f$), because the upward and downward half periods of H_t have the same effect on changing the transverse magnetic field of the nanoparticles in a spin valve sensor with a DC transverse bias field H_b [12]. In contrast to a DC magnetic excitation, an AC magnetic excitation will introduce electromagnetic interference (EMI) to the sensor signal, because the AC field will cause a time-varying magnetic flux in the measurement circuit which, in turn, generates an AC voltage as governed by the Faraday's law. This EMI can easily surpass the true signal from the nanoparticles and therefore extra care is needed in the AC mode detection.

For the nanoparticle detection demonstrated in this paper, we have chosen the in-plane DC mode for its EMI-free signal, the easiness of magnetic field alignment, and the simple measurement setup, for which only the transverse excitation field H_t is needed.

3. Magnetic nanoparticle detection

3.1. Sensor fabrication

The spin valve sensors with synthetic pinned layers and a magnetoresistance (MR) ratio of 11.3% were fabricated at submicron scale by e-beam lithography with a width of about 0.2 μm . Reducing sensor width from micron to submicron size will increase the effective field of the magnetic nanoparticles in the sensor and therefore enhance the sensor's detection sensitivity [7,11–13]. The sensor fabrication started with the spin valve thin film deposition on a 4-in. silicon wafer with the pinned magnetization (M_p) set in a selected direction. This selected direction will make M_p aligned in the width or transverse (y) direction of the patterned spin valve sensors, as illustrated in Fig. 1(a). e-Beam lithography (Hitachi HL-700F) was used to pattern sensor stripes on a submicron scale. The patterns were thereafter transferred into the spin valve thin film by timed ion milling. After striping off the e-beam resist, optical lithography (Electronic Visions 620 Aligner) was used to overlay leads pattern on the spin valve sensor stripes. Thick (300 nm) aluminum layer was then deposited on the wafer by ion beam deposition. Electrical leads and pads to the spin valve sensors were formed after lift-off. Fig. 3 shows (a) the optical micrograph of a fabricated spin valve sensor array of 60 sensors on a $\sim 7 \text{ mm} \times 8 \text{ mm}$ chip and (b) the scanning electron microscope (SEM) image of one submicron sensor in the array.

3.2. Magnetic nanoparticles

Monodisperse 16-nm Fe_3O_4 nanoparticles are used in the magnetic nanoparticle detection experiments [19]. The left inset in Fig. 4 shows a SEM image of the nanoparticles. At room temperature, the 16-nm Fe_3O_4 nanoparticles are superparamagnetic, as indicated by the magnetization curve shown in Fig. 4, which is measured with a vibrating sample magnetometer (VSM). Also shown in Fig. 4 is the fitting of the magnetization curve with the Langevin function weighted by a log-normal distribution of nanoparticle diameter [25,26]:

$$\frac{M_{\text{np}}}{M_{\text{s,mp}}} = \frac{\int_0^{\infty} L(\alpha) f(d_m) dd_m}{\int_0^{\infty} f(d_m) dd_m}, \quad (1)$$

where $L(\alpha)$ is the Langevin function with $\alpha = m_s H/kT$, d_m the effective magnetic diameter of nanoparticle, and $f(d_m)$ is the log-normal distribution of the magnetic diameter:

$$f(d_m) = \frac{1}{\sqrt{2\pi}d_m\sigma_d} \exp\left(-\frac{\ln^2(d_m/d_{m0})}{2\sigma_d^2}\right), \quad (2)$$

where d_{m0} is the median diameter and σ_d is the distribution width. The particle moment m_s is related to the magnetic diameter as $m_s = \pi d_m^3 M_s / 6$, where $M_s = 480 \text{ emu/cm}^3$ is the saturation magnetization of Fe_3O_4 , and the imperfection of a nanoparticle is represented by its reduced magnetic diameter with respect to its physical diameter. As we can see, the magnetization curve is well fitted by the weighted Langevin function, indicating the superparamagnetism of the nanoparticles. The particle diameter distribution is also plotted in the right inset of Fig. 4, for which $d_{m0} = 14.5 \text{ nm}$ and $\sigma_d = 0.25$. The mean diameter of the Fe_3O_4 nanoparticles is $\bar{d}_m = d_{m0} e^{\sigma_d^2/2} = 14.9 \text{ nm}$, close to the physical diameter of 16 nm as measured by transmission electron microscope (TEM) [19].

3.3. Experimental methods

In order to place magnetic nanoparticles on spin valve sensors, we developed a “bilayer lift-off” process to deposit the nanoparticles site-specifically. As schematically shown in Fig. 5(a), the bilayer lift-off process involves four steps: a bilayer of poly(ethylenimine) (PEI)/poly(methyl methacrylate) (PMMA) is sequentially coated onto the sensor surface (step 1). PEI is a functional polymer on which magnetic nanoparticles can self-assemble into a monolayer via ligand exchange between PEI and the surface surfactant (oleic acid) of the nanoparticles [27]. The PEI layer of $\sim 2 \text{ nm}$ thickness is dip-coated in chloroform solution. The e-beam resist layer PMMA is spin-coated on top of the PEI, and then the deposition areas are cleared by e-beam lithography (Raith 150) (step 2). After that, magnetic nanoparticles are applied in hexane solution onto the sensor surface. Some particles self-assemble on the exposed PEI layer in the deposition areas (step 3). Finally, the PMMA layer is stripped off with acetone and any nanoparticles on the PMMA layer are lifted off, leaving behind the self-assembled magnetic nanoparticles bound onto the PEI layer in the deposition area (step 4). For example, Fig. 5(b) shows a nanometer-scale dot pattern in the PMMA layer on a spin valve sensor and Fig. 5(c) shows 16-nm Fe_3O_4 nanoparticles deposited in such a dot area after lift-off of the PMMA layer. By varying the size, location of deposition area and the concentration of nanoparticles in solution, we gain control of the number and position of deposited nanoparticles, which enables us to perform quantitative detection of magnetic nanoparticles on spin valve sensors.

The magnetic nanoparticle detection is performed in the inplane DC mode with the magnetic excitation field H_t applied in the transverse direction (see Fig. 2(a)). In this work, *no bias field* H_b was applied for the submicron spin valve sensors due to their large shape anisotropy. The MR transfer curves, i.e., the sensor resistance versus the applied field H_t , are measured for the sensor with and without magnetic nanoparticles. The difference of the MR transfer curves indicates the presence and, further, the quantity of the captured nanoparticles.

We first patterned the nanoparticle deposition areas with varying sizes on a series of spin valve sensors on a chip like the one shown in Fig. 3(a). Then the sensor chip was brought to a probe station equipped with a homemade electromagnet and four tungsten probes. Driven by a DC power supply, the electromagnet supplied the magnetic excitation field H_t in the transverse direction of the spin valve sensors. The four tungsten probes were put in contact with the aluminum pads (see Fig. 3) to implement the four-probe measurement of the sensor resistance. A sense current of $I_{\text{sense}} = -0.1 \text{ mA}$ was sourced to a spin valve sensor by a Keithley 236 Source-Measure Unit where the negative current runs in the $-x$ direction (see Fig. 1(a)), while the voltage across the sensor was measured by a HP 3685 Multimeter. Both the magnetic field application and the sensor resistance measurement were controlled by a computer.

The 16-nm Fe_3O_4 nanoparticles, in hexane dispersion, were then applied onto the sensor surface. After drying, the PMMA layer was lifted off with acetone. As a result of the varying size of the nanoparticle deposition areas, the Fe_3O_4 nanoparticles were deposited in different quantities on different sensors. However, some sensors on the chip were intentionally skipped

during the e-beam lithography so that they have no deposition area and hence no nanoparticles on surface. The sensors with deposited nanoparticles are called “detection sensors” and the ones without nanoparticles “reference sensors”. During the nanoparticle deposition, the sensor chip remained immobile at the probe station. The MR transfer curves were measured again after applying the nanoparticles. Comparing these two transfer curves gives the resistance differences caused by the deposited nanoparticles $\Delta R = R_{\text{with}} - R_{\text{without}}$, where R_{with} and R_{without} are the sensor resistances with and without nanoparticles, respectively.

3.4. Detection results and discussions

Fig. 6(a) shows the resistance differences of three detection sensors (filled symbols) and two reference sensors (open symbols) as examples of the series of measurements. There are increasing numbers of Fe_3O_4 nanoparticles on detection sensors 1–3. Fig. 6(b and c) shows the SEM images of the detection sensors 1 and 2 with the deposited nanoparticles, respectively. The MR transfer curves before and after depositing the Fe_3O_4 nanoparticles of the detection sensor 3 are also shown in the inset of Fig. 6(a). Due to the small effect of the nanoparticles, the resistance differences do not look as apparent on the MR transfer curves as on the ΔR curves. The difference between the detection sensors and the reference sensors is readily noted in Fig. 6(a). Whereas the resistance differences of the reference sensors are almost flat around zero, the ΔR curves of the detection sensors show two peaks, one negative for positive H_t and the other positive for negative H_t . The appearance of the peaks is the result of two competing factors: the sensor sensitivity and the nanoparticle fields. When the applied field H_t decreases from saturating values, the field sensitivity of the sensor increases until it reaches the maximum at low field, as seen in the MR transfer curves. On the other hand, the polarization and therefore the field of the nanoparticles decreases with the decreasing H_t . Hence, the MR effect of the nanoparticles peaks at some field in between the saturation and zero. The opposite polarity of the peaks is due to the fact that the field from the nanoparticles is opposite to the applied field on the sensors.

Spaced by ~ 2 -nm thick PEI layer and ~ 5 -nm spin valve capping layer, the 16-nm Fe_3O_4 nanoparticles are about 7 nm above the free layer. At such a close distance, the magnetic stray field from the sensors will affect the polarization of the nanoparticles. The stray field comes from the ferromagnetic layers in the spin valve and from the sense current. The free layer stray field further interacts with the particle field during the detection. As a result of the influence of the sensor stray field, the positive and negative peaks in the ΔR curves need not be symmetric about zero applied field. Indeed, some shifts of the zero-crossing point are observed on the ΔR curves. The shifts are towards negative H_t in the $-y$ direction, indicating that the stray field from the spin valve sensors at zero applied field is positive, in the $+y$ direction. This positive sensor stray field may come from the negatively pinned moments, the free layer moment tilted negatively due to the interlayer coupling, and the sense current running in the $-x$ direction. In another set of detection experiments, we intentionally moved the nanoparticles away from the sensor top to the sensor side at a large distance (a few hundred nanometers). A much smaller shifting was then observed because of the reduced sensor stray field at the greater distance.

Fig. 7 shows the peak-to-peak resistance difference $\Delta R_{\text{p-p}}$ versus the number of deposited Fe_3O_4 nanoparticles. The particles are counted on the SEM images. The solid line is a linear fit to the data. The horizontal dashed line indicates the fluctuation range of reference sensor signals, and may be considered the noise level of this detection method. Each data point corresponds to one detection sensor on the chip. The three detection sensors (1–3 in Fig. 6(a)) are explicitly labeled. In Fig. 7 we notice that the sensor signal $\Delta R_{\text{p-p}}$ increases with the number of nanoparticles in a reasonably linear manner. The data scatter seen in Fig. 7 may be caused by the factors, such as the varying locations and distributions of nanoparticles on the sensors. Although the Fe_3O_4 nanoparticles have a narrow size distribution [19], they are not identical,

as seen in Fig. 4. This small distribution would also have an adverse impact on the sensor signal quantification, especially when the nanoparticle number is small as in this case. The sensor-to-sensor variation, such as physical dimension and field sensitivity, may contribute to the data scatter as well. Hence, it is foreseeable that the data scatter can be reduced by varying and counting nanoparticles on the same sensor for more realistic biodetection. In the current experiments, however, the deposited nanoparticles are strongly bound to the PEI layer so that the detection can only be performed once per sensor. Nonetheless, since these sensors are fabricated on the same chip and have closely similar properties, Fig. 7 represents to a large extent the sensor signal dependence on the nanoparticle quantity.

The fitting line in Fig. 7 intersects the reference signal level at 14 nanoparticles, which may be the minimum detectable number of nanoparticles in this detection scheme with a signal-to-noise ratio of ~ 1 . The inferred 14 or demonstrated 23 (on the detection sensor 1) Fe_3O_4 nanoparticles have a total magnetic moment of ~ 11 or 17 femu at the peaks of ΔR , respectively, which is one order of magnitude lower than the prior reported detection limit of one 250-nm magnetic label or 200 femu [6]. Some clustering is noted in the deposited nanoparticles, as seen in Fig. 6, which might reduce the particle moments because of the inter-particle magnetostatic interaction [9]. In real biodetection applications, the nanoparticle labels would be more isolated due to the steric repulsion of attached biomolecules and surface surfactants. As a result of this separation, the detection sensitivity will be further enhanced. It is conceivable that an even lower number (<10) of nanoparticles will be detectable by using more sensitive sensors, such as magnetic tunnel junctions [28,29], and higher moment particles, such as Co and Fe, or by using lock-in detection to narrow the noise bandwidth.

In these experiments, more than one reference sensors were used to make certain that the resistance differences caused by the nanoparticles can be well distinguished. These reference sensors were randomly selected on the sensor chip, and were not necessarily the nearest neighbors of the detection sensors. Our experiments indicated that reference sensor signals were consistent among one another, and seemed uncorrelated to the sensor location on the chip. This implies that only a small portion of sensors may be needed as the reference for a large-scale integrated sensor array, leading to a high utilization ratio of the spin valve sensors for biodetection.

4. Conclusions

Prototype GMR spin valve sensors have been designed and fabricated at a submicron scale for detection of magnetic nanoparticles intended as biomolecular labels in a magnetic biodetection technology. We demonstrate experimentally the detection of 16-nm superparamagnetic Fe_3O_4 nanoparticles in various quantities from hundreds down to a few tens by spin valve sensors at room temperature. A linear relationship between sensor signal and nanoparticle quantity has been found. These experiments provide a direct proof of the feasibility of using submicron spin valve sensors as highly sensitive biosensors and monodisperse superparamagnetic nanoparticles as magnetic labels for emerging magnetic biodetection technology.

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Biographies

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Robert L. White is a professor emeritus of electrical engineering and of materials science and engineering at Stanford University. He received his BA, MA, and PhD degrees in physics from Columbia University. He was on the scientific staff of Hughes Research Laboratories and of the General Telephone and Electronics Laboratory before joining the faculty at Stanford. He was chairman of the Department of Electrical Engineering from 1981 to 1987, and held the

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Nader Pourmand currently serves as senior research scientist in the Department of Biochemistry, Stanford Genome Technology Center, at Stanford University and leads the Bio-electrical and Pathogen detection laboratory. His main interest is to discover and develop novel methods for genetic analysis. In 1999, he obtained his PhD in experimental medicine and rheumatology from the Karolinska Institute in Sweden; and then joined the Stanford Genome Technology Center and developed pyrosequencing as a method for viral typing and for multiplex analysis of DNA samples. He has also been involved in Genotyping by a Co-Spotted Single-Base Extension Assay. In 2001, he invented a new chip-based DNA sequencing technology, Charge-Perturbation Signature (CPS): a new technique for DNA sequencing by utilizing charge-detection of extension. In addition to CPS he developed Bioluminescence Regenerative Cycle (BRC) system for nucleic acid quantification. He also refined and applied Molecular Inversion Probe for pathogen genotyping. He recently invented a Branch-Migration assay for short tandem repeats detection in forensic DNA fingerprinting.

Shan X. Wang currently serves as the director of the Center for Research on Information Storage Materials (CRISM), and is an associate professor in the Department of Materials Science & Engineering and jointly in the Department of Electrical Engineering at Stanford University. He is also with the Geballe Laboratory for Advanced Materials, and is affiliated with Stanford Bio-X Program. He received the BS degree in physics from the University of Science and Technology of China in 1986, the MS in physics from Iowa State University in 1988, and the PhD in electrical and computer engineering from the Carnegie Mellon University (CMU) at Pittsburgh in 1993. His current research interests lie in magnetic nanotechnologies in general and include bio-magnetic sensing, magnetic microarrays, novel magnetic nanoparticles, magnetoresistive materials and spin electronics, magnetic inductive heads and soft magnetic materials, as well as magnetic integrated inductors. He has published over 100 papers and holds 8 patents (issued and pending) on these subjects. He and Alex Taratorin also published a book titled "Magnetic Information Storage Technology" through Academic Press. Wang was among the inaugural group of Frederick Terman Faculty Fellows at Stanford University (1994–1997), and was an IEEE Magnetics Society Distinguished Lecturer (2001–2002).

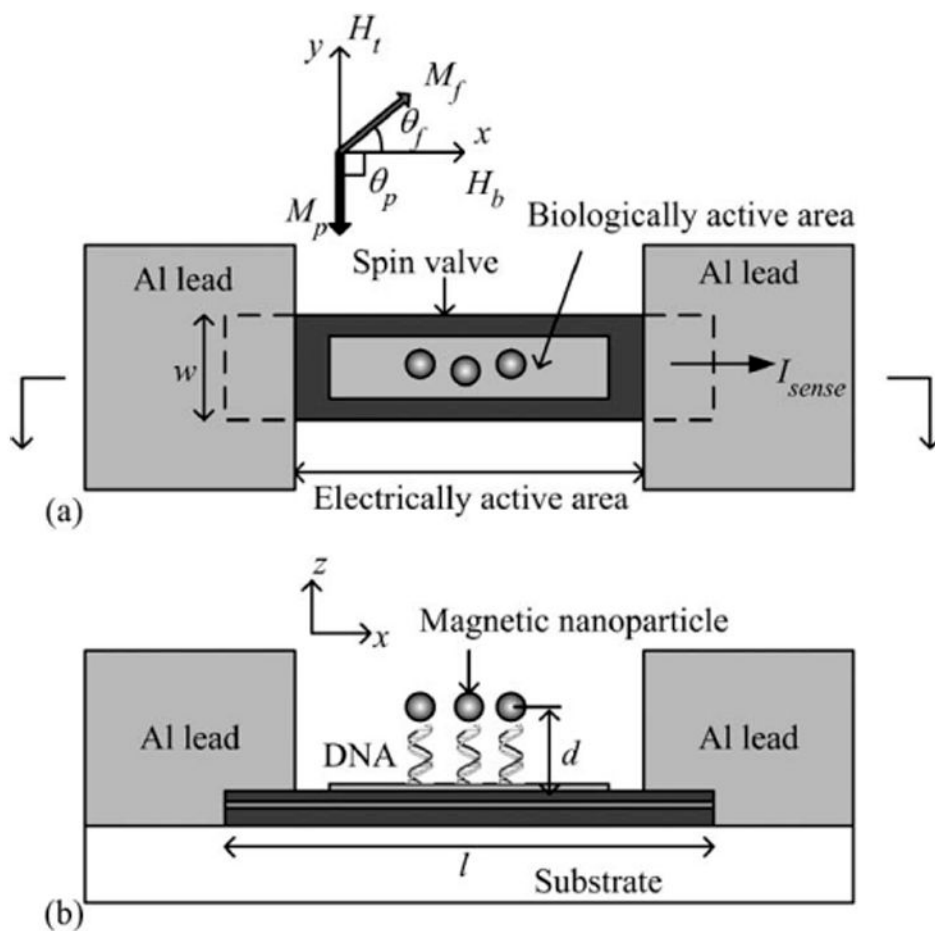


Fig. 1. Schematic illustrations of (a) the top view of a spin valve sensor and (b) its cross-section. A few magnetic nanoparticle labels are conceptually shown bound to the sensor through hybridized probe and target DNAs in the biologically active area. M_f and M_p are the free and pinned magnetization of the spin valve sensor, respectively. H_t and H_b are the applied magnetic excitation and bias fields, respectively.

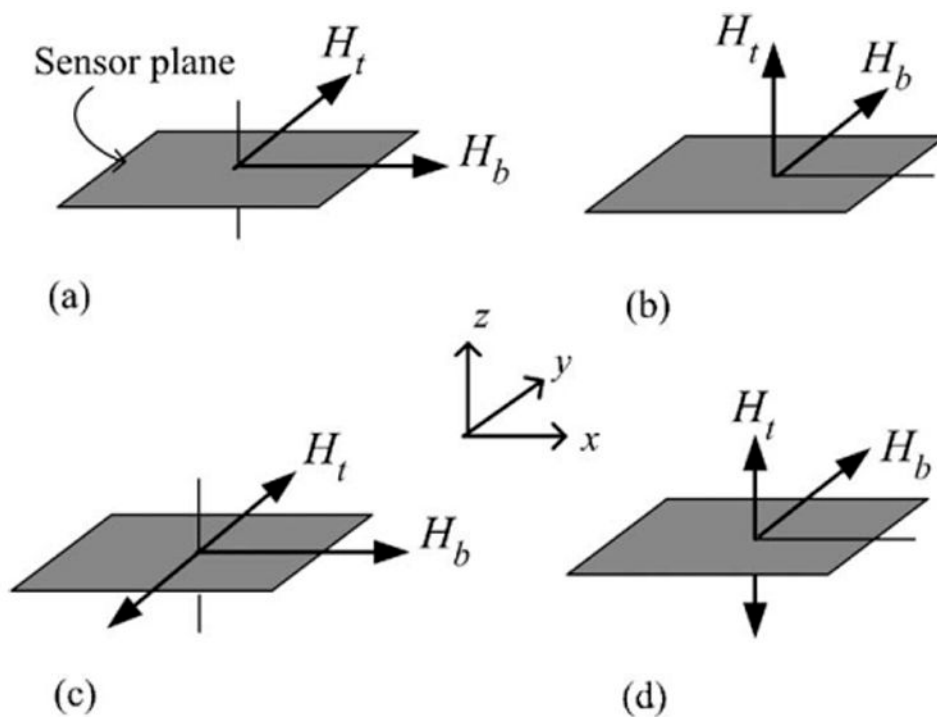


Fig. 2. Schematic illustrations of four nanoparticle detection modes: (a) inplane DC mode, (b) vertical DC mode, (c) in-plane AC mode, and (d) vertical AC mode, corresponding to the four combinations of the direction and form of the magnetic excitation field H_t . H_b is a constant DC bias field applied in the sensor plane. A double-head arrow indicates an AC field, while a single-head arrow indicates a DC field.

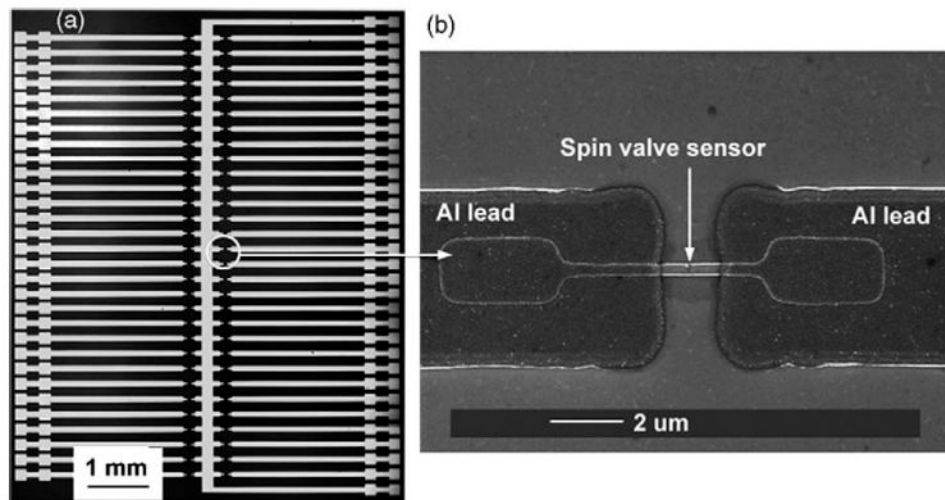


Fig. 3. (a) An optical micrograph of a fabricated spin valve sensor array of 60 sensors on a $\sim 7 \text{ mm} \times 8 \text{ mm}$ chip and (b) the scanning electron microscope (SEM) image of one submicron sensor in the array.

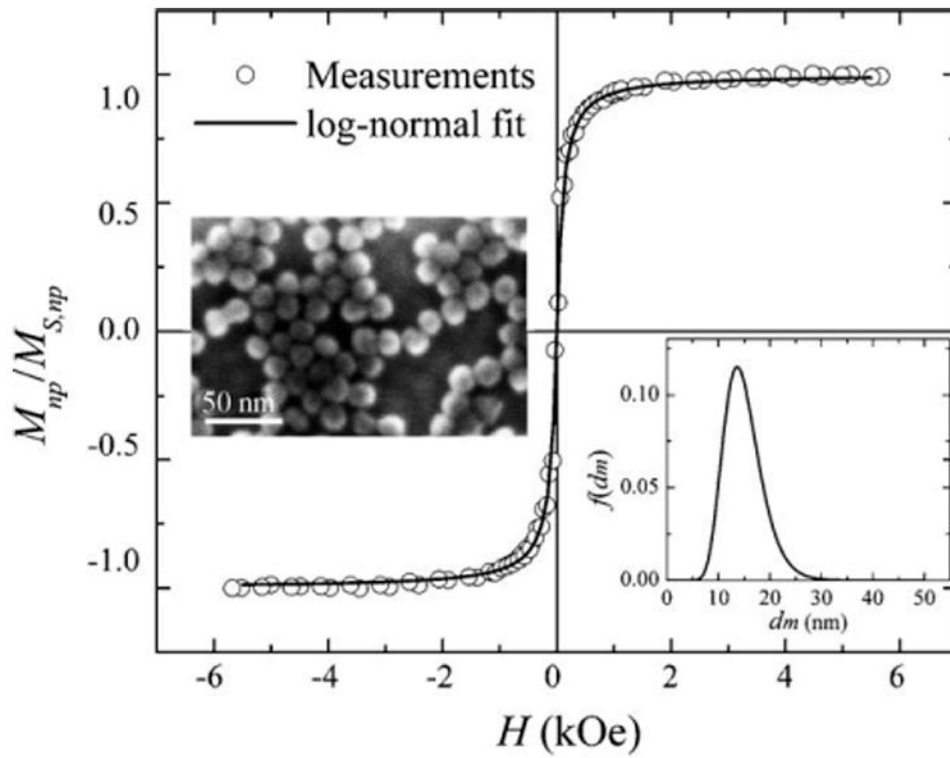


Fig. 4. Normalized magnetization curves of 16-nm Fe_3O_4 nanoparticles measured by VSM at room temperature. The left inset shows the SEM image of the nanoparticles. The right inset is the distribution of particle magnetic diameter obtained from the log-normal fitting to the magnetization curve.

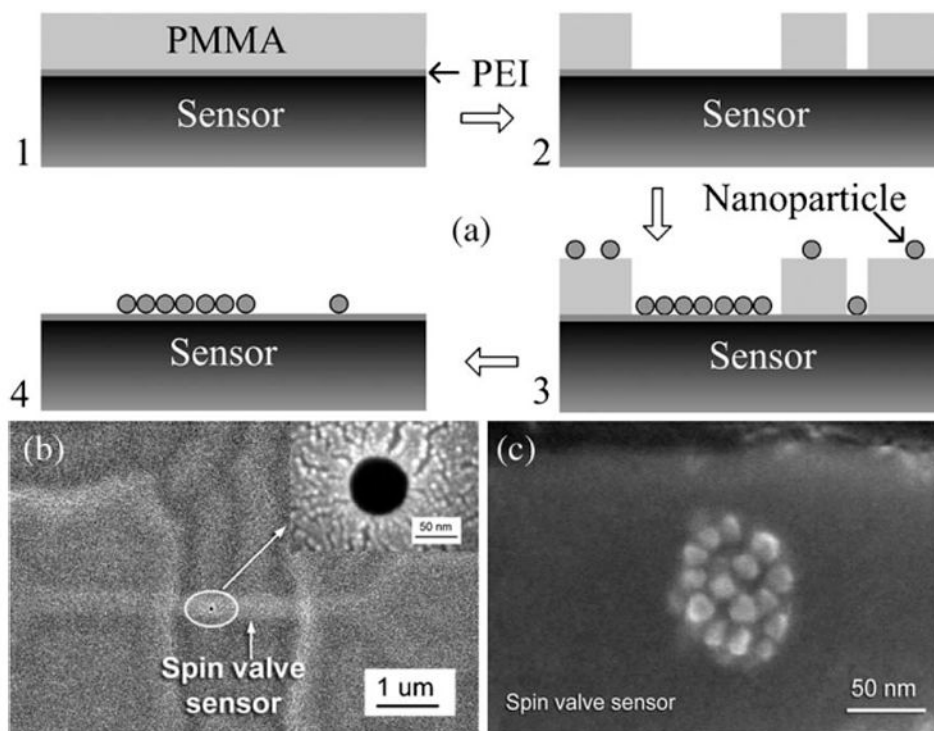


Fig. 5.

(a) Schematic bilayer lift-off process: (1) a PEI/PMMA bilayer is sequentially coated on a sensor surface, (2) deposition areas are created in the PMMA layer by e-beam lithography, (3) magnetic nanoparticles self-assemble on exposed PEI layer in the deposition areas, and (4) the resist layer and unwanted nanoparticles are lifted off by solvent. (b) SEM image of a nanometer-scale dot patterned in the PMMA bilayer on a spin valve sensor. Inset shows the close-up of the dot. (c) Sixteen-nanometer Fe_3O_4 nanoparticles deposited in such a dot pattern as shown in (b) after lift-off of the PMMA layer.

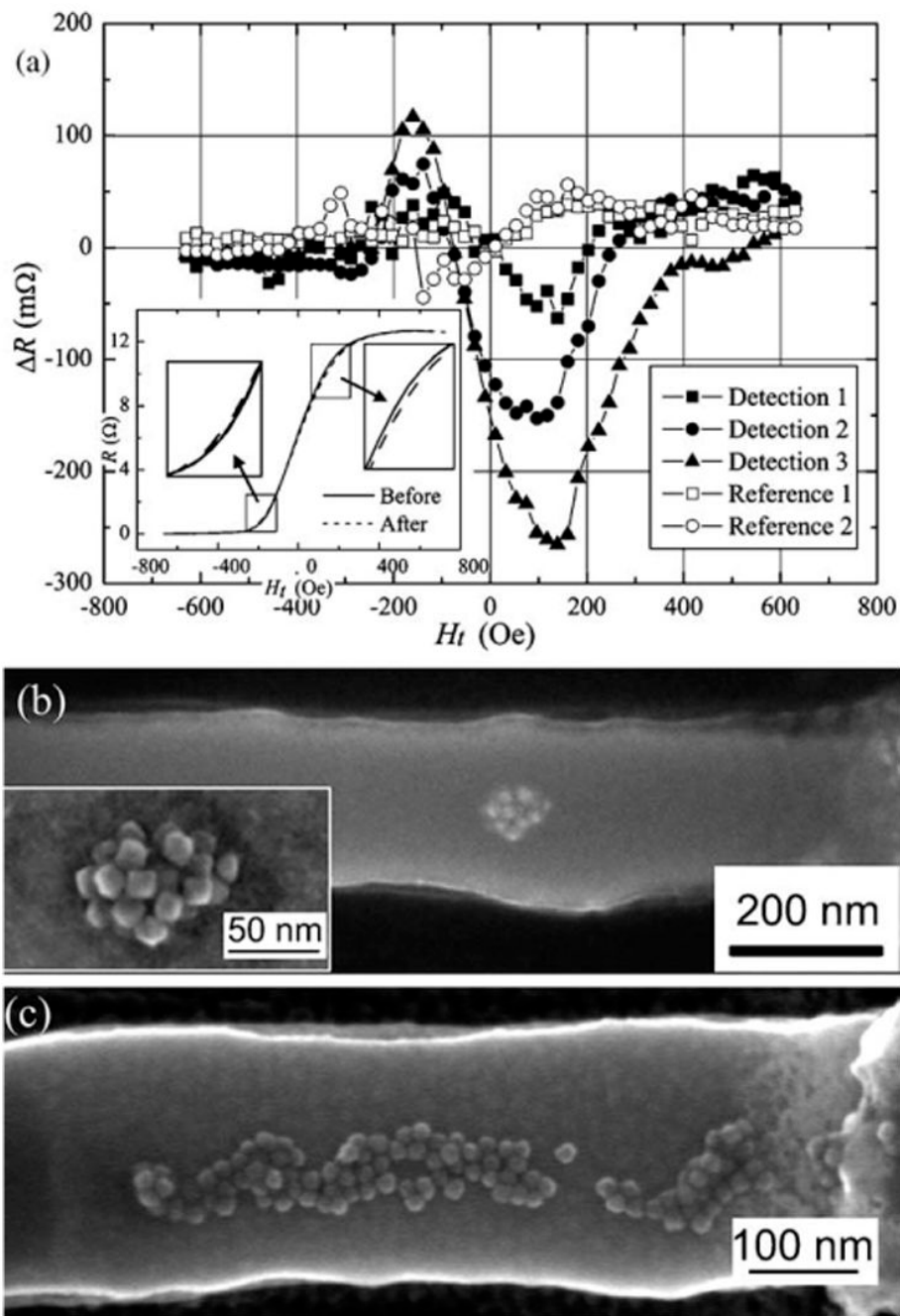


Fig. 6. (a) Resistance differences before and after depositing 16-nm Fe_3O_4 nanoparticles for three detection sensors (filled symbols) and two reference sensors (open symbols). The inset shows the MR transfer curves measured before (solid line) and after (dashed line) the nanoparticle deposition for the detection sensor 3. The three detection sensors have different numbers of the Fe_3O_4 nanoparticles. SEM images of (b) the detection sensor 1 with ~ 23 deposited Fe_3O_4 nanoparticles that are also shown in the inset at a higher magnification and (c) the detection sensor 2 with ~ 108 nanoparticles.

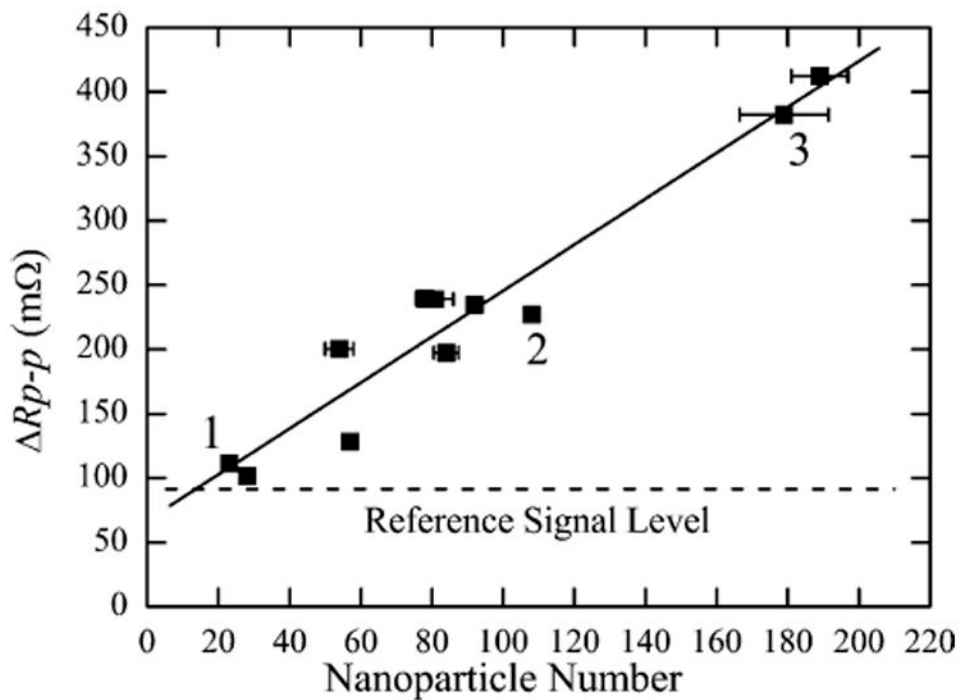


Fig. 7. The peak-to-peak resistance difference vs. the number of the deposited Fe₃O₄ nanoparticles. The three detection sensors in Fig. 6(a) are explicitly labeled. The solid line is a linear fit and the dashed horizontal line designates the average signal fluctuation range of the reference sensors.