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Magnetoresistive-based biosensors and biochips

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Over the past five years, magnetoelectronics has emerged as a promising new platform technology for biosensor and biochip development. The techniques are based on the detection of the magnetic fringe field of a magnetically labeled biomolecule interacting with a complementary biomolecule bound to a magnetic-field sensor. Magnetoresistive-based sensors, conventionally used as read heads in hard disk drives, have been used in combination with biologically functionalized magnetic labels to demonstrate the detection of molecular recognition. Real-world bio-applications are now being investigated, enabling tailored device design, based on sensor and label characteristics. This detection platform provides a robust, inexpensive sensing technique with high sensitivity and considerable scope for quantitative signal data, enabling magnetoresistive biochips to meet specific diagnostic needs that are not met by existing technologies.

Despite the success of fluorescence-based microarrays in biomedical research, the investigation of novel biochip platforms continues to be driven by the huge market potential for bio-detection systems offering unique advantages or reduced cost [1]. The technical challenges that still face fluorescence-based DNA microarray systems include a lack of quantitative analysis, the difficulty encountered in comparing data collected from different microarray platforms and the often prohibitively high cost of associated equipment such as array scanners. The detection platform suffers from high background-fluorescence from microarray substrates and would also benefit from the use of more stable biological labels – fluorescent labels are photo-sensitive and, hence, samples bleach when exposed to light. Since the late 1990s, magnetoelectronics [2] has emerged as one of several new platform technologies for biosensor and biochip development. This technology is based on the detection of biologically functionalized micrometer or nanometer-sized magnetic labels, using high-sensitivity microfabricated magnetic-field sensors. Although in its infancy, this technology offers high-sensitivity detection (to the level of a single molecular interaction, in principle), a stable labeling system, low magnetic background and cheap device components.

Magnetic field biosensor detection schemes

In the simplest, most direct case, the biomolecule to be detected (target or analyte) is magnetically labeled (immobilized on a magnetic label) and passed over an array of specifically patterned complementary or non-complementary (probe) molecules, which are immobilized over on-chip magnetic field sensors. The sensors detect the presence of the magnetic labels via a change in sensor resistance at a fixed sense current. The unbound target biomolecules are then washed away and residual sensor signals are obtained at sensor sites, where complementary magnetically labeled target- and surface-bound probe molecules have successfully interacted (Figure 1). An alternative method is based on a secondary detection step, performed after the interrogation of the probe array with the target molecules. In this method, the target molecules are labeled with a small biochemical label, such as biotin. Biotinylated target molecules, bound to complementary surface-bound probe molecules are then detected by

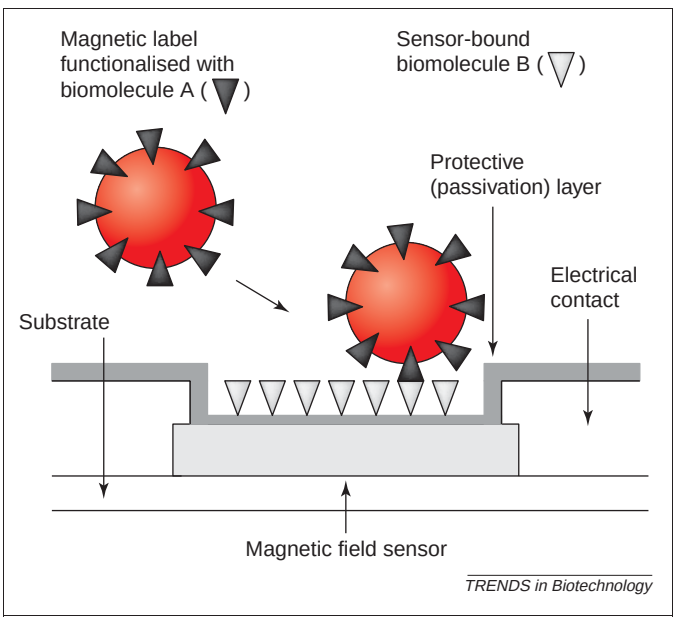

Figure 1. Simplified cross-sectional scheme for magnetically labeled biomolecule detection in biosensor development. The interaction of a magnetic label, functionalised with biomolecule A, with a magnetoresistive sensor-bound biomolecule B. The magnetic fringe field resulting from the magnetic moment of the label within an on-chip applied magnetic field changes the resistance of the sensor, resulting in a voltage signal (ΔV) at a sensing current (I). If this signal remains after the appropriate washing of the sensor surface, this indicates the detection of molecular recognition between biomolecules A and B.

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introducing magnetic labels functionalized with streptavidin, the complementary molecule to biotin. This approach has been used successfully in the detection of DNA–DNA hybridization on-chip, as illustrated in the scheme shown in Figure 2.

Basis of magnetoresistive biosensing

A biosensor can be defined as a 'compact analytical device or unit incorporating a biological or biologically-derived sensitive element integrated or associated with a physiochemical transducer' [3]. The biosensor research field is now vast [4,5], following a resurgence of interest in these devices in the 1990s, one of the contributing factors being the continued development of microfabrication technologies [6]. Magnetic field sensors, such as superconducting quantum interference devices (SQUIDS) and induction coils, have previously been used in biodiagnostics, but their application was limited by large size, low sensitivity and high power consumption. This has been overcome by the use of magnetoresistive (MR) materials. A change in the resistivity of a material due to a magnetic field is known as a magnetoresistive effect. This was first reported by Thomson in 1856, but it was not until the late twentieth century, that advances in solid state technology, such as the fabrication of extremely thin and soft MR ferromagnetic films (Ni80Fe20), allowed the widespread technological application of the principles involved. The discovery of antiferromagnetic interlayer exchange coupling [7] and the giant magnetoresistive (GMR [8]) effect in the 1980s opened up several applications for high-sensitivity magnetic nanostructures, including magnetic recording media, read heads and magnetic random access memory (MRAM). The anisotropic magnetoresistive effect (AMR) originates from the change in material resistance, which occurs when the magnetisation changes from parallel, with respect to the direction of current flow, to transverse. This effect is present in ferromagnetic alloys (i.e. NiFe, NiFeCo) and it forms the basis of single thin-film sensors such as the planar Hall sensor [9] and the AMR ring sensor [10]. The GMR effect is based on the spin-dependent interfacial and bulk scattering asymmetry that is found for spin-up and spin-down conduction electrons crossing ferromagnetic–nonmagnetic–ferromagnetic multilayer structures, where the parallel or antiparallel alignment of the ferromagnetic layers can be engineered. An applied magnetic field is used to change the relative orientation of the magnetisations of the two magnetic layers. When they are aligned, the electrical resistance of the structure is low. When the magnetisations are antiparallel aligned, the resistance is high. This is the basis of GMR sensors [11] and also spin valves [12], which are used in most computers as read heads measuring the fringe magnetic field created by magnetized regions on the track (bits).

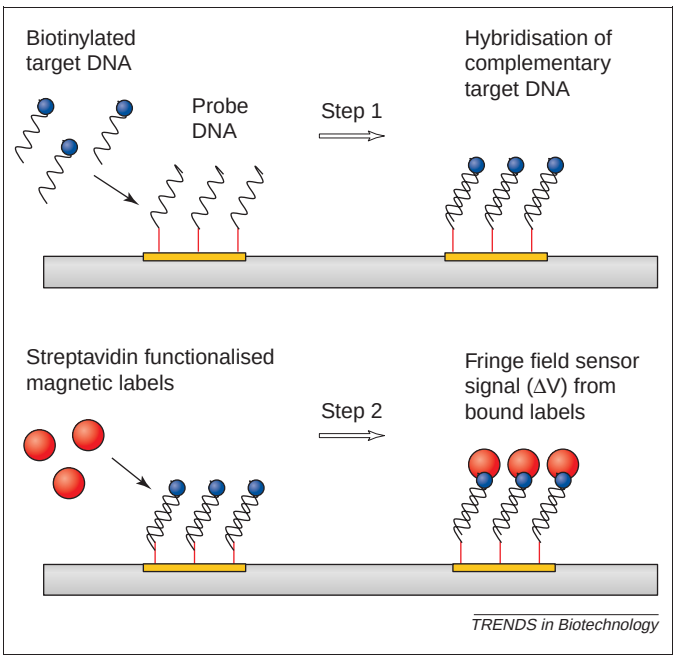


Figure 2. Simplified cross-sectional scheme for the use of magnetically labeled streptavidin to detect the location of pre-hybridised biotinylated DNA on-chip. A post-hybridization magnetoresistive DNA chip detection strategy. Step 1: probe DNA immobilised over on-chip magnetoresistive sensors are hybridized with biotinylated target DNA. Step 2: magnetically labeled streptavidin is used to detect the hybridized DNA by binding to the biotinylated hybridized target DNA. The magnetic fringe field of the labels is detected by the sensors.

Detecting magnetic labels

The labels used are non-remanent paramagnetic beads. The magnetic material within the label exists as small particles (usually iron oxide), having small random moments. The detection system relies on the alignment of these moments within the label to produce a measureable fringe field. A magnetic field is thus applied to the chip using a coil or horseshoe electromagnet. The electromagnet is used to induce an overall moment in the labels and also to center the sensors; that is, to bias the sensors within the linear regime of their magnetoresistive (magnetic field) response curve (MR curve). The direction of the applied field [which, for present sensor geometries, is either perpendicular to the chip surface or parallel to the surface (in-plane) and at a right angle to the sensor length] is dependent on the sensor used, although some sensors, for example, spin valves, can be used in either way [13,14]. The measurement schemes for a spin valve [13] or Hall cross sensor [9] are depicted in Figure 3a and b, respectively.

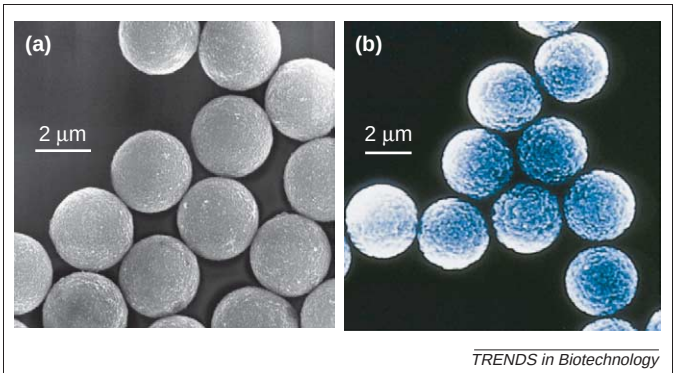


Figure 3. SEM images of magnetic microspheres. Scanning Electron Microscope (SEM) images of magnetic microspheres showing uniform size and shape: **(a)** 3 μm Micromer®-M, image courtesy of Micromod and **(b)** 2.8 μm M280 Dynabeads, image courtesy of Dynal Biotech.

Magnetic labels

Magnetic labels or carriers, also referred to as microspheres, microbeads and nanoparticles, have found wide-ranging scientific and clinical application in biotechnological and biomedical research, most notably in the areas of bioseparations, molecular biology and drug delivery [15]. The most important characteristics of magnetic labels that are used in biosensor or biochip devices are size and shape, chemical and magnetic composition, surface properties, stability and ease of chemical functionalisation for the immobilisation of biomolecules. The biochemical functionalisation of the magnetic label used depends on the intended application of the device; the size and magnetisation of the label depends on the size and sensitivity of the sensors incorporated and the necessity to prevent the blockage or fouling of the microfluidic components of the device during analysis. The size of the label used can vary from a few microns in diameter to a few nanometers, so size will be used here as a simple means of classification.

Magnetic microspheres

The larger labels ($\sim$1–3 μm diameter) have been the most widely studied in preliminary detection experiments using different types of MR sensor. They are easily observed and, hence, enumerable using non-specialized light microscopy techniques. They may also be manufactured in a uniform size and shape using preparative methodologies such as cone-filling (Micromer®-M, Micromod; http://www.micromod.de) or core-shell techniques (Dynabeads® M280, Dynal; http://www.dynal.no). Furthermore, despite the fact they have a lower percentage magnetic composition ($\sim$15%) in comparison to magnetic nanoparticles, their increased volume results in a higher magnetic moment per label in an applied magnetic field, allowing distinct detection signals at the single-label level [13]. Their uniform size and shape (Figure 4) also allows for quantitative signal data with linearity between the signal and the number of labels detected for a defined sensing area. The disadvantages of micron-sized labels are the high mass of the label in relation to the biomolecules tethering the label to the sensor surface and the large diameter of the label, hindering high-density label binding across the sensor surface.

Magnetic nanoparticles

Smaller nanometer-sized labels with a high magnetic (iron-oxide) content (70–85%) offer a solution to these problems; the smaller size allows for increased density of label binding across the sensor surface. Unfortunately, at present, most commercially available magnetic nanoparticle product samples contain particles with heterogeneous size (for example. 200–400 nm) and shape (non-spherical), thus hindering quantification. In addition, their high resultant magnetisation and anisotropy for their volume in an applied magnetic field may lead to rapid clustering (single particles aggregating to form groups). Permanent clustering of labels, which cannot be remedied using a discriminatory magnetic force [16] applied to the chip or on-chip washing cycles, can lead to exaggerated positive signals because non-biologically bound labels may remain attached to biologically bound labels. Finally, despite their higher magnetic percentage composition, magnetic nanoparticles as detectable labels acquire a smaller magnetic moment than a magnetic microsphere in an equivalent applied field (e.g. $\sim 10^{-13}$ and $\sim 10^{-12}$ emu, respectively, at 15–20 Oe [13]. Consequently, smaller labels require progressively more sensitive sensors and measurement systems. The majority of labels that have been used to date have been iron-oxide (magnetite or maghemite)-based, although transition metal containing nanoparticles (for example, NiFe or CoFe) offering higher magnetisation are now being studied [14].

Progress in magnetoresistive biosensing

Magnetoresistive-based devices [17], such as large GMR sensors and spin valves, have been used to detect a variety of commercially available magnetic microbeads [9,10,13,14,18–20] and magnetic nanoparticles [13,14,20], as a basis for biochip development. The detection of molecular recognition (the interaction of complementary or affinity-linked biomolecules) has been shown using two types of streptavidin functionalised magnetic labels (micrometer and nanometer sized) and sensor-bound biotin [21] and the biotin–streptavidin binding couple has been used as a means to detect hybridised DNA on-chip [19,22,23] (Figure 2). Recent developments include on-going improvements in device design and demonstrations of the reliability of these techniques in real-world biological applications. The types of MR sensors that are presently under investigation include large GMR sensors [17] and spin valves [13,14], anisotropic magnetoresistive ring (AMR) sensors [10] and Hall crosses [9]. Promising candidates for future work include planar Hall effect sensors [24] and magnetic tunnel junctions [25]. Other magnetic field sensors used in biodiagnostic devices include inductive coils [26,27] and SQUIDS [28,29].

Magnetoresistive detection signals

The MR sensors that are used to detect magnetic labels differ in size, geometry, structure and functionality, resulting in different levels of sensitivity, dynamic range and signal:noise ratios for the detection of a magnetic label of the same size and magnetisation. An initial attempt to compare and contrast the different types of sensor used has been performed [30], although this has been hampered by the different developmental measurement systems employed under different experimental conditions. The sensor signal obtained (change in voltage) depends on the inherent magnetic sensitivity of the sensor used and certain physical parameters such as the label:sensor size ratio, the magnetic moment of the label, the distance between the label and the sensing layer, the sense current, and whether or not signal amplification techniques are used. The moment of the label, which depends on the magnetic composition and content, is related to the applied magnetic field. The moment increases with an increase in applied field, with a linear response, until the field becomes saturating and the moment no longer increases [20]. Consequently, reported sensor signals for single micron-sized magnetic labels vary from <1 nV to >100 μV,

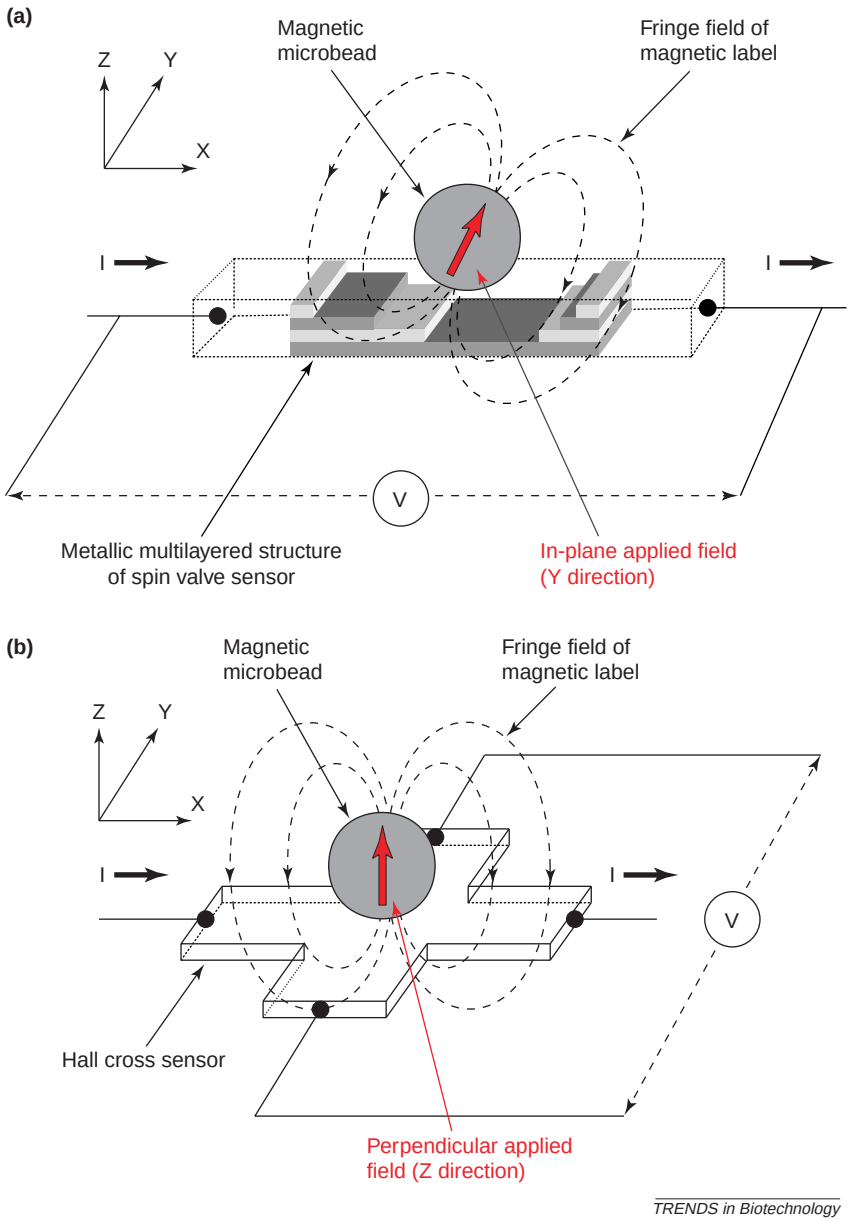


Figure 4. Magnetic label signal measurement for spin valve and Hall cross sensors. Signal measurement principle for two types of magnetoresistive sensor with different structure and geometry. **(a)** Multilayered metallic spin valve sensor, based on the GMR effect; the label fringe field is detected with a sensor that is biased using an in-plane applied magnetic field **(b)** Single-layer Hall cross sensor, based on the AMR effect; the label fringe field is detected with a sensor that is biased using an applied magnetic field perpendicular to the sensor surface. (b) is adapted from [6], courtesy of P.A. Besse and re-printed with the kind permission of the American Institute of Physics.

Table 1. Magnetoresistive sensors and magnetic labels used to date in magnetoresistive detection platforms

Sensor Type	Size (μm)	Label size	Sensitivity	Range	Molecular Recognition?	Refs
Spin valve	2 × 6	2 μm	1	1–6	Yes	[13,20,21]
		0.5–1.5 μm		1–10	No	[20]
		1 μm		1–15	No	[20]
		250 nm	10 s	10–100 s	Yes	[13,18,21]
		100 nm	100 s	1000 s	No	[20]
		50 nm	1000 s	1000 s	No	[20]
Spin valve	1 × 2.5–3 × 12	2.8 μm	1	<10	No	[14]
		11 nm (Co)	>1000 s	–	No	[14]
AMR ring	5 (d)	4.3 μm (NiFe)	1	–	No	[10]
Hall sensor	2.4 × 2.4	2.8 μm	1	–	No	[9]
GMR spiral	70 (d)	2.8 μm	200	>1000	Yes	[19]
GMR strip	5 × 80	2.8 μm	1	<100	Yes	[17,22]
GMR serpentine	200 (d)	2.8 μm	10	>1000	Yes	[23]

depending on the sensor and the system set-up (Table 1). Sensor sensitivity and dynamic range can be expressed magnetically or biologically. The magnetic sensitivity can be considered as the minimum magnetic moment that can be detected or the smallest magnetic label of a particular magnetic composition. In biological terms, the sensitivity can be considered as the smallest number of detectable biomolecular interactions (Table 2 and Box 1) or the lowest target biomolecule concentration that is required to produce a binding signal. The dynamic range can also be expressed in both ways, but is most simply expressed as the range of the number of labels that can be detected by a single sensor or the range of target biomolecule concentrations that can be quantitatively distinguished by a single sensor. In brief, to date, it appears that small spin valves ($1 \times 3\ \mu m^2$ or $2 \times 6\ \mu m^2$) offer the highest sensitivity with good signal:noise ratios, large GMR sensors ($\sim 100 \times 100\ \mu m^2$, serpentine or spiral geometry) offer increased dynamic range, planar Hall sensors offer ease of fabrication and AMR rings provide the ideal geometry for single microsphere detection.

Magnetoresistive biochips

The biochip consists of an arrangement of single or multiple biosensing elements in a series of sensing zones, designed and fabricated on-chip to facilitate multi-probe or multi-analyte-based detection. The first aspect of chip design is the layout. The chip dimensions (usually mm scale) are defined and the available chip surface is used as efficiently as possible, in such a way as to maximize the active sensing area within each sensing zone, to incorporate appropriate reference sensors and yet avoid electrical, magnetic or thermal crosstalk between sensors or on-chip structures. A differential sensor set-up uses a reference sensor in a Wheatstone bridge architecture to enable thermal and electrical (mains) drift compensation between a biologically active sensor and an biologically inactive sensor. The design of a first generation biochip, based on spin valve sensors, is shown in Figure 5. This chip was fabricated with 12 pairs of sensors, used for both single and differential sensor measurements [21]. These structures were fabricated on 3′ silicon wafers, using microelectronic processing techniques under cleanroom conditions. The structures are defined using laser lithography and sensor materials were deposited by sputtering [13,20,21]. The integrity of the on-chip structures was then assessed via microscopic inspection and electrical resistance measurements.

Box 1. The basis of a theoretical calculation of DNA molecules detected per magnetic label

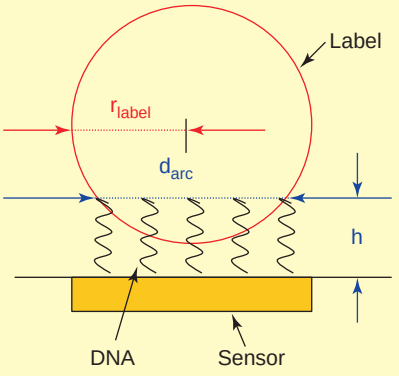
The number of DNA molecules attached to a magnetic microsphere (diameter, 'd' and radius, 'r') surface (via a biotin–streptavidin linkage) interacting with a sensor surface was calculated on the basis of an arc area of the sphere in contact with the sensor. The arc area is estimated according to the length of the DNA molecules interacting with the sensor-bound DNA molecules, and the calculation of the number of DNA–DNA interactions is based on several given assumptions.

Basis for calculation

- Surface area of label = $4\pi r^2$
- Surface area of arc in contact with sensor = $2\pi rh$, based on height (h).

Assumptions

- Label is spherical
- Label has uniform size and shape
- Uniform distribution of streptavidin molecules
- 1–4 target DNA molecules bound per streptavidin molecule
- 'h' for 50mer oligonucleotide DNA probe molecule = ~34 nm (18 nm for oligo + 12 nm target DNA overlap + ~4 nm for cross-linkers).



A protective (passivation) layer was deposited over the chip structures to prevent corrosion of the chip surface by fluids applied during the chemical and biological reactions. This material (substrate) should also provide ease of surface functionality for the immobilisation of biomolecules and, ideally, should show low non-specific adherence of magnetic labels, polymers, proteins and nucleic acids. Sputtered silicon dioxide (~2000 Å thick) was used in combination with aqueous phase biochemistry without problems [21]. Only the electrical contacts at the outer edges of the chip remain free of the passivation material.

Table 2. Theoretical calculation data for DNA–DNA molecular interactions detected per magnetic label

Magnetic label	d (nm)	r (nm)	sa (nm^2)	No. strep.	No. DNA	sa_{arc} (nm^2)	No. DNA_{arc}
Micromer®-M	2000	1000	1.3×10^7	$\sim 77 \times 10^3$	77×10^3	2.1×10^5	1300
Latex-M	400	200	5.0×10^5	$\sim 3.8 \times 10^3$	3.8×10^3	4.3×10^4	320
Nanomag®-D	250	125	2.0×10^5	~500	500–2000	2.7×10^4	70
Nanomag®-D	130	65	5.3×10^4	~70	70–280	1.4×10^4	20
Nanomag®-D-spio	100	50	3.1×10^4	~10	10–40	1.1×10^4	3
Nanomag®-D-spio	50	25	7.9×10^3	~2*	2–8	5.3×10^3	1

Theoretical calculation of the number of probe–target DNA interactions detected using a magnetically labeled target DNA, binding to a 50mer oligonucleotide sensor-bound probe. Estimation of the DNA-DNA interactions between the arc of a spherical magnetic label interacting with the sensor surface assuming that one biotinylated DNA target molecule is bound to each label–bound streptavidin molecule within the arc. The type of label, diameter (d), radius (r), surface area of the label (sa), number of streptavidin molecules per label (No. strep), potential number of target DNA molecules (No. DNA), surface area of the arc (sa_{arc}) and the potential number of target DNA molecules within the arc interacting with the sensor-bound probe molecules (no.DNA_{arc}). Calculations are based on the assumptions listed in Box 1.

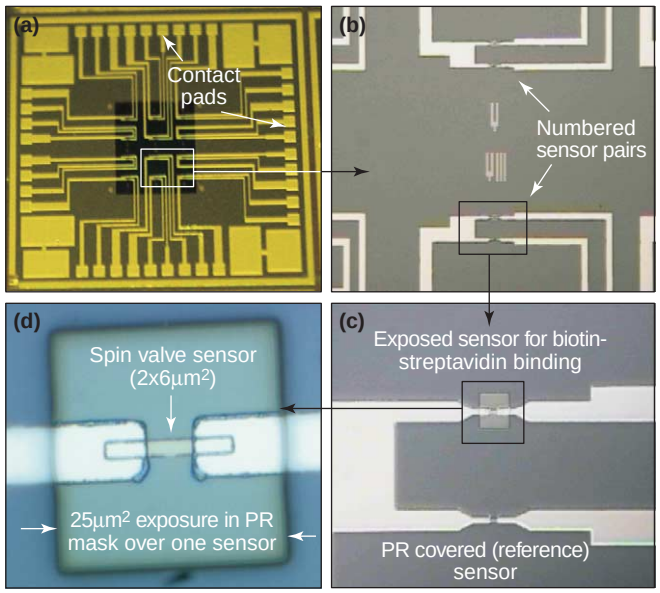


Figure 5. A magnetoresistive-based biochip designed with 12 pairs of spin valve sensors, used for single or differential signal measurements. **(a)** The 8 × 8 mm chip has the sensor pairs fabricated in the central area, covered with photoresist (PR) mask, the sensor connections running to contact pads arranged around the outer edges of the chip. **(b)** Each sensor pair has one input line and two separate output lines. **(c)** One sensor of each pair is exposed to on-chip biochemistry (active sensor) via an exposure in the photoresist. **(d)** A single 2 × 6 μm^2 spin valve with contacts within a 25 × 25 μm^2 exposure in the PR mask. Adapted from [10] and re-printed with the kind permission of Elsevier.

The wafer is diced into individual chips and each one is mounted in a chip carrier for easy connection to hardware. The contacts are wire-bonded to the pads, which are then protected by a silicon gel, or microfluidics are used to control fluid-flow across the chip [31]. The microelectronic fabrication techniques for other sensors and chips can vary, as described in the references in Table 1.

Biological considerations

The MR sensing technique is versatile in that it can potentially be used to detect and analyse magnetically labeled nucleic acids, proteins, whole cells or microorganisms. DNA chips and protein chips for immunoassay are perhaps the most obvious areas of immediate application. The choice of DNA chip developed depends largely on the nature of the sensor signals obtained. Gene expression profiling (measuring a variation in gene expression) requires a chip that is dedicated to the comparison of upregulated or downregulated genes in a sample in relation to a control gene. This requires strictly quantitative analysis, that is, discernable and reproducible differences in the sensor signals obtained at different DNA probe sites on the chip. In this case, sensor units with a broader dynamic range (the range of the number of magnetic labels detected per sensor) would be advantageous. DNA chips for the detection of DNA mutation sites [most importantly, the detection of single nucleotide polymorphisms (SNP's)] may require only qualitative ('yes or no') signals, but require the ability to discern between very small differences in the sequence of two DNA strands. Such a chip would preferably have many sensing zones; there are often very high numbers of SNP's or other mutations associated with common diseases. Thus, many probes are required. This provides a greater technical challenge in MR chip development, because a large number of sensors per chip requires efficient multiplexing. Further challenges to be addressed include the treatment of real samples (cell/tissue) before the detection step. This requires careful consideration of the appropriate labeling methodology and the correct approach to be used in the detection.

The biotin–streptavidin binding model was used by several groups, using different MR sensors, to demonstrate the basic proof of principle of the MR chip [17,19,21,22]. This model was chosen because streptavidin has four binding sites for biotin and the two exhibit very high binding affinity and high binding strength [32]; furthermore, biotin can also be used as a biochemical label for nucleic acids and proteins, thus enabling the use of streptavidin functionalised magnetic labels to detect biotinylated target molecules, as discussed. This approach might also help to prevent steric hinderance of the biological interaction of magnetically labeled target molecules with a sensor-bound molecule. Post-hybridisation detection of DNA on MR chips has been demonstrated [19,22,23], but this also requires a time-consuming conventional on-chip hybridisation step. More recently, we have used magnetic

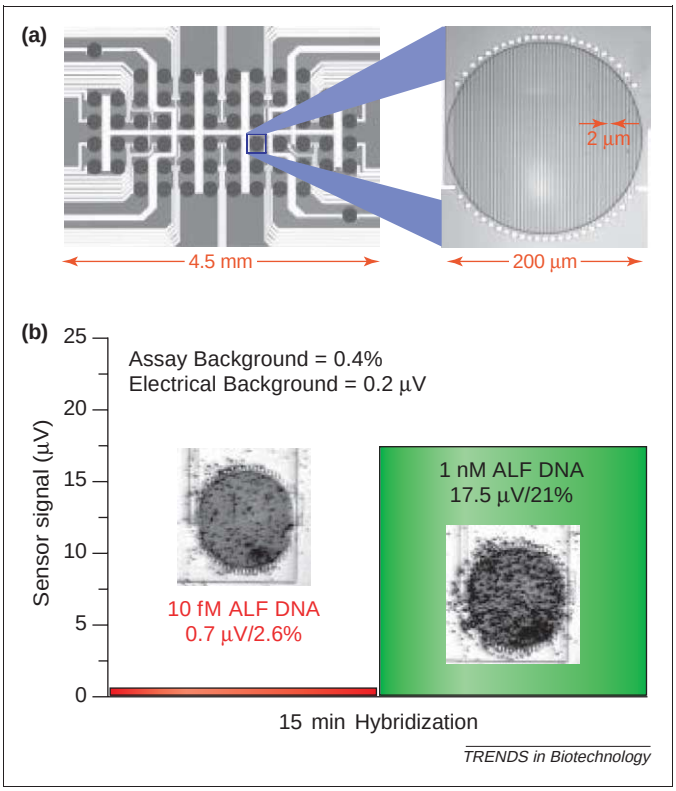


Figure 6. The bead array counter (BARC III) biochip and typical DNA detection data. The BARC III chip, based on 64 serpentine GMR elements and two reference elements. **(a)** The chip layout with an insert showing one circular serpentine sensor area designed to give a high percentage sensing area beneath one DNA probe spot (approximately 100 × 100 μm^2). **(b)** Voltage signals obtained for two different DNA concentrations, expressed as a voltage change and a percentage sensor bead coverage using streptavidin functionalised 2.8 μm M280 Dynabeads (Dynal) to detect probe DNA that is pre-hybridised with biotinylated target DNA. (a) and (b) combined from [12], courtesy of C.R. Tamanaha and re-printed with the kind permission of Kluwer Academic Publishers.

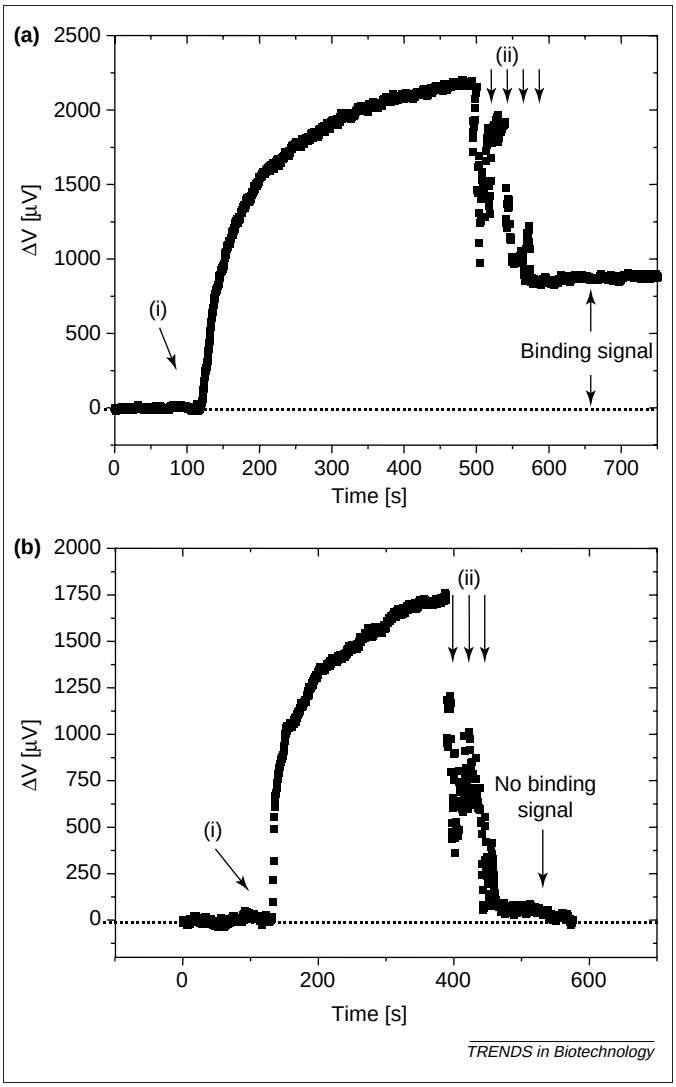


Figure 7. Real-time magnetoresistive detection of a cystic fibrosis-related DNA target. Post hybridisation detection of a wild-type DNA sequence, in which the most common CF-related mutation (del508) occurs. A 50mer oligonucleotide probe was immobilised over $2 \times 6\ \mu m^2$ spin valve sensors and hybridised with **(a)** biotinylated complementary target DNA and **(b)** biotinylated non-complimentary DNA. Hybridised DNA was then detected via the introduction of 250 nm streptavidin-functionalised magnetic nanoparticles toward sensor saturation (i), followed by washing of the sensors (ii). The resulting hybridisation signal of 0.9–1.0 mV for sensor-bound probe hybridised complementary target DNA (a) corresponds to 90–100 nanoparticles or ~50% sensor coverage with labels. Sensor-bound probe hybridised with non-complimentary target DNA gave no hybridisation signal. Target DNA concentration ~200 nM, on-chip biotinylated hybridised DNA and streptavidin functionalised nanoparticle binding, performed in 5 μl of 100 mM phosphate buffer, pH 7.2.

field generating current lines [13] in combination with spin valve sensors for the rapid and simultaneous detection of hybridisation on-chip using magnetically labeled target DNA. This is achieved by using the magnetic fields that are generated by tapered current lines to rapidly focus magnetically labeled target DNA at sensor sites. In this case, DNA hybridisation and detection can be performed using an MR biochip in minutes, using very low target DNA concentrations (in principle, small numbers of DNA molecules, Table 2).

Protein chips offer unique challenges, mostly associated with the complex secondary and tertiary structure of proteins, resulting in a higher degree of precision required for successful recognition. Despite this, we believe that MR biochips have strong potential applications in high sensitivity protein-based micro-assays and anticipate publication in this area in the near future.

Prototype devices and specific applications

Two generations of prototype bead array counters (BARC, II and III) have used large GMR sensors (strip and serpentine) and a bead-capture technique [17,22,23,33]. The BARC III chip employs a 64-sensor element array arrangement, illustrated in Figure 6a, designed to enable quantitative binding signals with respect to the percentage label coverage (Figure 6b). These prototypes, which are the most advanced to date with respect to a final device, have been tailored toward the DNA chip application; more specifically, toward the detection of biological warfare agents such as *Bacillus anthracis*, *Yersinia pestis*, *Brucella suis*, *Francisella tularensis, Vibrio cholerae*, *Clostridium botulium*, *Campylobacter jejuni* and Vaccinia virus. In parallel, a first generation of MR biochips based on small spin valve sensors [13,18,20,21] are presently being applied to the development of diagnostic biochips for cystic fibrosis (CF), a complex genetic disease. Figure 7 presents the spin valve MR data for post-hybridisation detection of a CF-related DNA target sequence using an appropriate probe versus a non-specific, non-complementary target. In addition, both spin valves and large GMR sensors are currently being used in the development of MR-based micro-immunoassays.

Summary

The MR-based detection of single micron-sized magnetic labels has been shown clearly and supported by theoretical magnetic modelling [13,34]. Single magnetic nanoparticle (~100 nm) detection is anticipated in the near future, paving the way for simpler single-molecule detection applications. In terms of DNA chips, the MR approach offers a stable labeling system with low-cost components in what will ultimately provide compact, user-friendly detection devices. At this stage in their development, MR biochips do not compete with DNA microarrays in terms of the number of on-chip/on-slide DNA probe elements. Besides low cost, the most immediate advantage of MR biochips may lie in combination with magnetic field-generating chip structures [13,18,35], which can be used to rapidly focus magnetically labeled biomolecules at sensor sites and, hence, vastly reduce the time of hybridisation or other molecular recognition processes. The use of electric fields for this purpose has already been commercialized by Nanogen (NanoChip™; http://www.nanogen.com), although this biochip platform also employs costly fluorescence-based detection. At present, MR biochips represent a young, but rapidly expanding research area, promising high sensitivity, high quality quantitative molecular recognition detection data for a variety of biological applications.

References

1 Blohm, D.H. and Guiseppi-Elie, A. (2001) New developments in microarray technology. *Curr. Opin. Biotechnol.* 12, 41–47
2 Prinz, G.A. (1998) Device physics-magnetoelectronics. *Science* 282, 1660–1663
3 Turner, A.P. (2000) Tech. Sight. Biochemistry. Biosensors – sense and sensitivity. *Science* 290, 1315–1317

4 Harsanyi, G. (2000) *Sensors in Biomedical Applications*, CRC Press
5 Eggins, B.R. (2002) *Chemical Sensors and Biosensors*, John Wiley & Sons
6 Hierlemann, A. *et al.* (2003) Microfabrication techniques for chemical/biosensors. *Proc. IEEE* 91, 839–863
7 Grünberg, P. *et al.* (1986) Layered magnetic structures: evidence for antiferromagnetic coupling of Fe layers across Cr interlayers. *Phys. Rev. Lett.* 57, 2442–2445
8 Baibich, M.N. (1988) Giant magnetoresistance of (001)Fe/(001)Cr magnetic superlattices. *Phys. Rev. Lett.* 61, 2472–2475
9 Besse, P.A. *et al.* (2002) Detection of a single magnetic microbead using a miniaturized silicon Hall sensor. *Appl. Phys. Lett.* 80, 4199–4201
10 Miller, M.M. *et al.* (2002) Detection of a micron-sized magnetic sphere using a ring-shaped anisotropic magnetoresistance-based sensor: a model for a magnetoresistance-based biosensor. *Appl. Phys. Lett.* 81, 2211–2213
11 Hardman, U. ed. (1996) *Magnetic Thin Film and Multilayer Systems: Physics, Analysis and Industrial Applications*, Springer Series in Material Science
12 Freitas, P.P. *et al.* (2000) Spin valve sensors. *Sens. Actuat. A* 81, 2–8
13 Graham, D.L. *et al.* (2002) Single magnetic microsphere placement and detection on chip using current line designs with integrated spin valve sensors: biotechnological applications. *J. Appl. Phys.* 91, 7786–7788
14 Li, G. *et al.* (2003) Detection of single micron-sized magnetic bead and magnetic nanoparticles using spin valve sensors for biological applications. *J. Appl. Phys.* 93, 7557–7559
15 Häfeli, U. *et al.* eds (1997) *Scientific and Clinical Applications of Magnetic Carriers*, Plenum Press
16 Lee, G.U. *et al.* (2000) Implementation of force differentiation in the immunoassay. *Anal. Biochem.* 287, 261–271
17 Baselt, D.R. *et al.* (1998) A biosensor based on magnetoresistance technology. *Biosens. Bioelectron.* 13, 731–739
18 Lagae, L. *et al.* (2002) On-chip manipulation and magnetization assessment of magnetic bead ensembles by integrated spin-valve sensors. *J. Appl. Phys.* 91, 7445–7447
19 Schotter, J. *et al.* (2002) A biochip based on magnetoresistive sensors. *IEEE Trans. Magn.* 38, 3365–3367
20 Ferreira, H.A. *et al.* (2003) Biodetection using magnetically labeled biomolecules and arrays of spin valve sensors. *J. Appl. Phys.* 93, 7281–7286
21 Graham, D.L. *et al.* (2003) High sensitivity detection of molecular recognition using magnetically labeled biomolecules and magnetoresistive sensors. *Biosens. Bioelectron.* 18, 483–488
22 Miller, M.M. *et al.* (2001) A DNA array sensor utilizing magnetic microbeads and magnetoelectronic detection. *J. Magn. Mater.* 225, 138–144
23 Tamanaha, C.R. *et al.* (2001) Magnetic method for DNA detection on an arrayed solid state device. In *Micro Total Analysis Systems* (Ramsey, J.M. and van den Berg, A., eds), Kluwer Academic Publishers, pp. 444–446
24 Montaigne, F. *et al.* (2000) Development of magnetoresistive sensors based on planar Hall effect for applications to microcompass. *Sens. Actuat.* 81, 324–327
25 Gallagher, W.J. *et al.* (1997) Microstructured magnetic tunnel junctions. *J. Appl. Phys.* 81, 3741–3746
26 Richardson, J. *et al.* (2001) A novel measuring system for the determination of paramagnetic particle labels for use in magneto-immunoassays. *Biosens. Bioelectron.* 16, 1127–1132
27 Richardson, J. *et al.* (2001) The use of coated paramagnetic particles as a physical label in a magnetic immunoassay. *Biosens. Bioelectron.* 16, 989–993
28 Katsura, S. *et al.* (2001) Development of a new detection method for DNA molecules. *Supercond. Sci. Tech.* 14, 1131–1134
29 Lee, S. *et al.* (2002) Magnetic gradiometer based on a high transition temperature quantum interference device for improved sensitivity of a biosensor. *Appl. Phys. Lett.* 81, 3094–3096
30 Rife, J.C. *et al.* (2003) Design and performance of GMR sensors in the detection of magnetic microbeads in biosensors. *Sens. Actuat.* 107, 209–218
31 Nguyen, N-T. and Wereley, S. (eds) (2002) *Fundamentals and Applications of Microfluidics*, Artech House
32 Pérez-Luna, V.H. *et al.* (1999) Molecular recognition between genetically engineered streptavidin and surface-bound biotin. *J. Am. Chem. Soc.* 121, 6469–6478
33 Edelstein, R.L. *et al.* (2000) The BARC biosensor applied to the detection of biological warfare agents. *Biosens. Bioelectron.* 14, 805–813
34 Tondra, M. *et al.* (2000) Model for detection of immobilised superparamagnetic nanosphere assay labels using giant magnetoreistive sensors. *J. Vac. Sci. Technol. A* 18, 125–129
35 Lee, C.S. *et al.* (2001) Microelectromagnets for the control of magnetic nanoparticles. *Appl. Phys. Lett.* 79, 3308–3310