Detection of Cystic Fibrosis Related DNA Targets Using AC Field Focusing of Magnetic Labels and Spin-Valve Sensors

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A spin-valve sensor biochip was used to detect cystic fibrosis related DNA targets for the purpose of developing an affordable diagnostic chip. The strategy used was based on the ac field focusing of magnetically labeled target DNA at sensor sites using U-shaped current lines. U-shaped spin-valve sensors, fabricated within the line structures, detected in real-time the hybridization of DNA targets to complementary DNA probes, previously immobilized onto the chip surface. Hybridization occurred in relatively short times (15–30 min) in comparison with conventional hybridization approaches (3 to 12 h). Statistical data on detection signals for single probe and multiprobe experiments was obtained, showing a significant difference between complementary binding signals and noncomplementary and background ones.

***Index Terms*—Biomedical transducers, DNA, magnetic particles, magnetoresistive devices, medical diagnosis.**

I. Introduction

THE use of magnetoresistive sensors for biological applications has been generating an ever-increasing interest from laboratories and companies in the last few years.

The main interest so far has been the detection of DNA hybridization using magnetoresistive biochips, and this has been accomplished by using two detection approaches. In the first one, designated as post-hybridization detection, biotin-labeled DNA targets are incubated with a DNA probe functionalized chip surface for hybridization to occur. At a later stage, streptavidin-functionalized magnetic labels are incubated with the chip, binding to the hybridized targets. Hybridization detection is achieved through the sensing of the stray fields created by the labels using the magnetoresistive sensors. The hybridization process usually takes from 3 to 12 h, as targets need to diffuse passively in solution to find their complementary DNA probe on the surface. This approach has been used at the Naval Research Laboratory, at INESC—MN, and at the University of Bielefeld [1]–[5], but is unsuitable for applications where a fast response is required or desired, such as biological warfare agent identification, microbial pathogen detection, or clinical diagnostics during a medical doctor's appointment.

Recently, INESC-MN has followed a second approach designated as magnetic field assisted hybridization and detection. In this case, magnetically labeled DNA targets are transported and concentrated at DNA probe functionalized sensing regions by use of specially designed current lines. The proximity of target and probe increases the rate at which hybridization occurs. Previously, tapered current lines were fabricated [6] and used to focus labels at small $2 \times 6\ \mu\text{m}^2$ spin-valve sensors and hybridization times of less than 5 min were observed and detected [7]. These sensors can only detect small numbers (1–200) of 250 nm magnetic particles bound to the surface. In order to achieve a higher sensing dynamic range and higher biological sensitivity a new magnetic focusing system was developed, such that labels were focused in larger sensing areas. This system is based on the use of a combination of ac and dc magnetic fields created by an external electromagnet and by on-chip U-shaped current lines. An oscillating magnetic field gradient is created, focusing labels inside the U-shaped line structures and enabling magnetic field assisted hybridization [8], [9].

This paper reports on the detection of cystic fibrosis related DNA targets using the ac field focusing method and integrated U-shaped spin-valve sensors. Statistical data from an array of sensors was obtained with the purpose of showing the applicability of the magnetoresistive biochip platform for fast genetic disease diagnostics.

II. Experimental Method

U-shaped spin-valve sensors of 2.5 μm × 80 μm (full sensing length) were deposited on 3-in Si/Al_2O_3 (500 Å) wafers by an ion beam deposition system. The spin-valve stack has the structure Ta 20 Å/NiFe 30 Å/CoFe 25 Å/Cu 26 Å/CoFe 25 Å/MnIr 60 Å/Ta 30 Å/TiW(N) 150 Å. As-deposited spin valve coupon samples showed a magnetoresistance ratio (MR) of ~7.5% and, when patterned, the sensors showed a MR of $7.40 \pm 0.06\%$ ($\pm$ represents standard deviation), a sensitivity in the linear regime of $0.130 \pm 0.005\%$/Oe, and a resistance of $750 \pm 30\ \Omega$. The spin-valves were defined inside U-shaped aluminum current line structures 3000 Å thick, 10 μm wide, 120 μm full length, with

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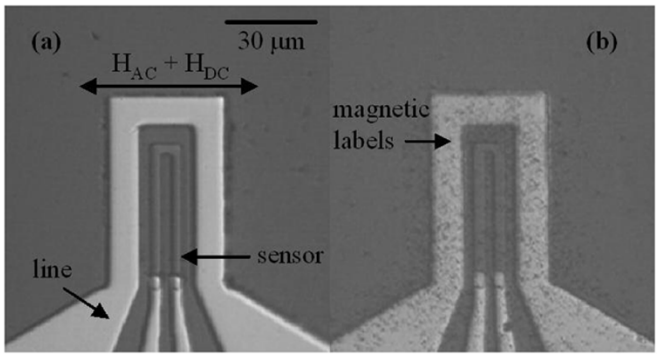

Fig. 1. U-shaped spin-valve sensor and current line. Photographs taken after ac focusing magnetic field assisted hybridization experiments: using (a) noncomplementary or (b) complementary target DNA. In (b), 250 nm magnetic labels bound to the surface are observed.

a spacing between the arms of the line of 17 μm; thus corresponding to an area of $\sim$1000 μm^2 where magnetic labels are focused and detected (Fig. 1).

Aluminum 3000 Å thick leads were evaporated to contact sensors to wire-bonding pads and a 3000 Å thick oxide layer (1000 Å Al_2O_3+ 2000 Å SiO_2) was used to protect the chip against chemical corrosion and to provide a suitable surface for DNA probe functionalization. Individual chips containing an array of sensors and associated U-shaped lines were diced and wire-bonded to 40-pin chip carriers.

Single-stranded 50-mer DNA probes and targets were synthesized by MWG Biotech with end modifications (3'-thiol for the probes and 3'-biotin for the targets). They were designed to correspond to genes that have been found to be either up- (*rpl29*) or down-regulated (*asah*) in cystic fibrosis related cell-lines versus healthy cell lines by preliminary microarray analysis of gene expression [10].

Probes were immobilized over the chip surface using a protocol described in [7]. Targets were magnetically labeled by incubating biotinylated DNA targets with streptavidin-functionalized 250 nm diameter nonremanent magnetic particles (Micromod Nanomag-D) using a protocol similar to the one described in [7]. Final target concentrations in solution were smaller than 83 fmol/μl ($\sim$1.3 pg/μl).

During experimentation an array of 6 or 16 sensors was monitored in real-time by applying a 1 mA sense current sequentially to each sensor and recording an ac voltage change with a general-purpose interface bus controlled lock-in amplifier. Sensor multiplexing was achieved with a CMOS analog multiplexer controlled by a PC board. An in-plane ac excitation field of 13.5 Oe rms at a frequency of 30 Hz was applied, together with an in-plane 24 Oe dc bias field, in the spin-valve sensing direction (Fig. 1). Sensor output was measured at 30 Hz with a constant time $\tau = 300$ ms (frequency bandwidth of $1/4\tau \sim 0.83$ Hz). The excitation fields were created by a horseshoe electromagnet with a NiFe core powered by a function generator in parallel with a dc power supply. The electromagnet was placed over the chip-carrier.

The ac field focusing of magnetic labels was achieved by applying currents of 25 to 40 mA rms at a frequency of 0.2 Hz through the U-shaped line structures in combination with the 24 Oe dc bias field.

TABLE I
MAGNETIC FIELD ASSISTED HYBRIDIZATION: SINGLE PROBE

Step	Detection signal (μV rms) [Normalized] (%)	
	rpl29 target (complementary)	*asah* target (non-complementary)
Saturation	1515±95 [23.70±2.13]	1620±149 [25.60±1.77]
Wash 1	335±170 [5.30±2.79]	17±11 [0.28±0.18]
Wash 2	256±127 [4.06±2.10]	15±7 [0.24±0.12]

Detection signals for 6 monitored sensors using a single probe (*rpl29*) functionalized on the chip surface. Values in brackets correspond to particle detection signals normalized to sensor output without particles. ± represents standard deviation.

Magnetic field assisted experiments using the ac field focusing method proceeded as follows: 1) small volumes ($\sim$20 μl) of the solutions of magnetically-labeled DNA targets were added to the chip; 2) ac field focusing was applied for 10 or 20 min; 3) particles were left to completely settle down over the sensor for 5 or 10 min (saturation); 4) the chip was washed with 100 mM phosphate buffer, pH 7, to remove unbound labels (wash 1); and 5) the chip was washed again with a more stringent buffer, 100 mM phosphate buffer, pH7, containing 150 mM NaCl, to remove weakly or nonspecifically bound labels (wash 2).

III. RESULTS AND DISCUSSION

Table I shows statistical results obtained for ac field focusing magnetic field assisted hybridization experiments (10 min ac focusing at 40 mA rms +5 min settle time) where the chip was functionalized with a single probe (*rpl29*) and interrogated with either complementary (*rpl29*) or noncomplementary (*asah*) magnetically-labeled targets (six sensors were monitored).

In Table I, saturation signals correspond to particle detection signals when a large number of particles is settled over the sensor (step (3) in the experimental protocol). It is observed that saturation signals are comparable in the two experiments and that deviation in signals is less than 10%. Binding detection signals (signals after washing) on the other hand are significant different: $\sim$20$\times$ higher in the case of the complementary target than in the noncomplementary case, indicating that hybridization occurred in the first case but was negligible in the latter. After the second, more stringent, wash the complementary binding signal is smaller, which is consistent with the removal of weakly or nonspecifically bound targets. Notice that hybridization signals can vary $\sim$50% among the different sensors. A number of cumulative issues are thought to be responsible for the deviation: nonuniform probe immobilization; nonuniform hybridization efficiency; nanoparticle size variation and clustering; nonuniform magnetically labeled target solution dispensing on chip; nonuniform label attraction to the U-shaped lines; and sensor resistance and sensitivity variation.

The values within brackets presented in the table are the detection signals normalized (in %) to the sensor voltage output without particles (the presence of particles results in an ac

TABLE II
MAGNETIC FIELD ASSISTED HYBRIDIZATION: SINGLE TARGET

Step	Detection signal (μV rms) [Normalized] (%)		
	rpl29 probe (complementary)	*asah* probe (non-complementary)	no probe (background)
Saturation	1227±82 [18.20±1.26]	1163±100 [19.58±1.50]	1107±45 [17.42±0.92]
Wash 1	313±140 [4.61±2.01]	42±17 [0.70±0.28]	48±10 [0.75±0.14]
Wash 2	112±27 [1.67±0.42]	10±2 [0.17±0.03]	15±11 [0.24±0.17]

Detection signals obtained using a single target (*rpl29*) and different probes. 16 sensors where monitored: *rpl29* probe (7 sensors); *asah* probe (6 sensors); no probe (3 sensors). Values in brackets correspond to particle detection signals normalized to sensor output without particles. ± represents standard deviation.

voltage drop). This normalization accounts for differences in sensor resistance and sensitivity.

Photographs taken after these experiments were performed are shown in Fig. 1. It is observed that the sensor surface was clean when the noncomplementary target was used. On the other hand, when the chip was interrogated with the complementary target, magnetic labels were bound to the surface of the sensor and of the U-shaped line, indicating that hybridization occurred. Notice that few particles were bound outside the focusing structure, although the surface was also functionalized with probe.

Table II shows statistical results obtained for ac field focusing magnetic field assisted hybridization experiments (20 min ac focusing at 25 mA rms +10 min settle time) where the chip was functionalized with *rpl29* and *asah* probes and interrogated with *rpl29* magnetically labeled targets (16 sensors were monitored in total).

In this experiment, it is also observed that saturation signals are similar among different sensors. Complementary binding signals are now $\sim$7 to 10$\times$ higher than noncomplementary or background signals, which are comparable and indicate that the system has good biological specificity. Furthermore, higher specificity is expected if washing procedures are improved.

IV. CONCLUSION

This paper has shown first statistical data on the detection of cystic fibrosis related DNA targets. Although, deviations on the hybridization signals can be $\sim$50%, the detection signals are at least 3$\times$ (in the worst observed case) to $>$ 10$\times$ higher than noncomplementary or background signals.

In conventional microarray gene expression analysis, a fluorescence signal (coming from a hybridized DNA spot) of intensity $<$ 0.5$\times$ or $>$ 2$\times$ that of a signal obtained for a control sample, represents a down- or an up-regulated gene, respectively. It means that at least a two-fold signal distinction from background and nonspecific binding is necessary. Although hybridization signal deviations still need to be studied and minimized, it seems that already the magnetoresistive biosensing platform can be competitive with or superior in performance to conventional assays when analysis of the expression of few genes is required, such as in a diagnostic method.

In addition, the ac focusing magnetic field assisted hybridization method can provide results within 30 min to 1 h, opening the possibility to have, in the future, a fast genetic diagnosis during a visit to the doctor.

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