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A novel silicon based mags-biosensor for nucleic acid detection by magnetoelectronic transduction

Maria Eloisa Castagna [a,*], Salvatore Petralia [a], Maria Grazia Amore [a], Emanuele Cappello [b], Angela Beninato [c], Valentina Sinatra [b], Salvatore Baglio [c], Sabrina Conoci [a,*]

[a] STMicroelectronics, Stradale Primosole 50, 95121 Catania, Italy
[b] Distretto Micro e Nano sistemi, Ottava Strada, 5 -Zona Industriale, 95121 Catania, Italy
[c] DIEEI, Universita' degli Studi di Catania, Viale Andrea Doria, 95120 Catania, Italy

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ABSTRACT

We developed a novel silicon biosensor based on magnetoelectronic transduction (MAGS) for nucleic acid detection. The mags-biosensor is a planar device composed by a primary micro-coil, and two secondary coils which produce a differential voltage due to the induced magnetic field. The presence of magnetic material over one of the secondary coils causes variations of induced magnetic field density that in turn results in a total output voltage different from zero. The voltage variation, therefore, is a measure of the amount of magnetic material present in the active zone. A device sensitivity of 5.1 mV/ng and a resolution of 0.008 ng have been observed. The biosensor also presents a micro-heater and a thermal sensor respectively to set and read-out the chip temperature: this aspect enables the device to be used for several biochemical applications that need temperature control and activation such for example nucleic acid amplification (real-time PCR), antigen- antibody detection (immune-assay) and SNP detection.

1. Introduction

Since its invention in the early 90s [1] real-time PCR has become an indispensable tool in many fields of molecular diagnostics, including determination of viral or bacterial loads in clinical samples, identification of germs in food, diagnosis of tumors, gene expression analysis, or forensic analyses [2]. The spread diffusion is due to the real time DNA amplification read out, thereby avoiding time-consuming post-PCR analysis.

Moreover, real-time PCR instruments currently on the market are based on complex and delicate optical components that lead to rather large, fragile and costly instruments, thereby restricting their use to specialized laboratories of analysis. The development of new miniaturized solutions for nucleic acids (DNA, RNA) analysis in portable and easy to use format (point-of-care), is one of the most important research pervasion of molecular analysis in the decentralized centers (hospitals, physician office).

Future point-of-care (PoC) molecular-level diagnosis requires advanced biosensing systems that can achieve high sensitivity and portability at low power consumption levels, all within a low price-tag for a variety of applications based on nucleic acids analysis such as epidemic disease control, biohazard detection, and forensic analysis. Several efforts have ears on the development of integrated optical components with high sensitivity to detect low fluorescent signals from small sample volume in order to miniaturize actual commercial instruments [3].

The interest in miniaturized biosensors is driving also the research efforts in finding electrical transduction mechanism. Biosensors development would be easier to produce (if made on Si-based devices and technologies) and to integrate in complex circuitries [4–6].

Magnetically labeled biosensors have been proposed as a promising solution for the miniaturization and integration and as candidate to potentially eliminate or augment the optical instruments used by conventional fluorescence-based sensors [7,8]. Magnetic detection methods require, in fact, minimal instrumentation, low electrical power supply and easily integration of both sensor element and circuitry in the same chip [8,9]. With respect to other types of markers (radioactive, enzymes, fluorophores, luminescent etc.), magnetic ones have many potential advantages: lower cost, higher stability, absence of toxicity, etc. Most of the applications employing magnetic beads devices have been focus on proteins detection (immune-assay). To the best of our knowledge there are only few examples in the literature that employ magnetic detection for nucleic acids [10,11].

In this paper, the proposed device, is basically a microtransformer constituted by a primary coil and two secondary coils connected in a differential configuration: the magnetic field generated by the

* Corresponding authors at: Stradale PrimoSole 50, 95125 Catania, Italy.
E-mail addresses: mariaeloisa.castagna@st.com (M.E. Castagna), sabrina.conoci@st.com (S. Conoci).

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primary coil, induces, at the secondary coils, voltages equal and opposite [9,12].

The presence of magnetic material over one of the secondary coils causes variations of induced magnetic field density that involves a total output voltage different from zero. The voltage variation, therefore, is a measure of the amount of magnetic material present in the active zone. The differential configuration has been chosen to filter undesired environmental noise: just one of the two secondary coils is made sensitive to the magnetic bead suitably functionalizing its surface.

The approach allows then an optimization of the device in terms of sensitivity [9,12].

In addition, biomolecules fixed to magnetic nanoparticles can be easily localized and manipulated by suitable magnetic fields.

The biosensor also presents a heater and a thermal sensor respectively to set and read-out the chip temperature: this aspect enables the device to be used for several biochemical applications that need temperature control, i.e. nucleic acid detection (Real Time PCR), immune-assay (antigen-antibody detection), SNP detection, host-guest detection etc.

The aim of this paper is to demonstrate the feasibility of integrated inductive magnetic sensors and their sensitivity to small amounts of magnetic beads, which is a promising characteristic for the future applications of the proposed approach to high-sensitivity, low-cost, portable biosensors. Also thermal components have been characterized and calibrated and its behavior in working regime analyzed.

2. Materials and method

2.1. Device fabrication

The thermal-magnetic sensor has been fabricated using conventional standard CMOS process technology. A high resistive silicon substrate (4500 Ω cm) has been chosen in order to minimize the loss of the magnetic field generated. Furthermore a thick silicon stoichiometric oxide (2 μm) has been thermally grown to electrically insulate the device from the substrate.

A first Aluminum (Al) metallization has been then sputtered in order to realize the thermal components, both heater and sensor. The thickness of the first metallization is 1 μm. A silicon oxide layer has been deposited and a mechanical planarization process has been implemented to avoid surface morphology: the residual oxide thickness in metal strips is 0.3 μm. After the planarization a dry etch has been performed to connect the first and the second metallization where necessary (i.e. on interconnection tracks).

The primary winding has been realized in the second metal layer (Al). The Al thickness (2.5 Am) has been chosen in order to regulate the current circulating in the primary coil and then the magnetic field generated. Again a silicon oxide has been deposited and planarized and then a third metal (Al) sputtered (1 μm) to define the secondary coils.

The dielectric layers thicknesses between metals have been maintained thinner than 0.3 μm to minimize effects of capacitive couplings.

Also metal 2 and metal 3 are connected through a vertical dry etch where necessary. Finally metal 3 is planarized through a deposited silicon oxide layer with residual thickness 0.3 μm and a 700 nm silicon nitride layer. The nitride, as passivation layer, has been selected to be roughness to chemical process necessary for the surface funzionalization required by application. The residual oxide and the nitride are removed just on the region to contact the device (see Fig. 1).

Fig. 2 shows the device layout with and without the heater ad thermal sensor (cyan layer).

The dimension of the whole die is 2400 μm × 2400 μm. The secondary coils present opposite winding sense; therefore, the resulting output voltage, which is the difference between the voltages across the secondary coils, is zero when no magnetic particles are present. Working

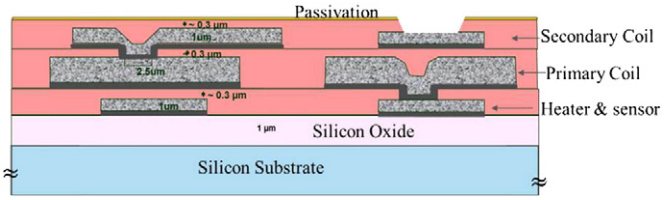

Fig. 1. Schematic cross-section of the device.

frequencies well below the RF range have been assumed as design constraint in order to simplify the readout circuitry that would be less sensitive to external interferences than in the case of high-frequency circuits. Moreover, from the point of view of the application as a biosensor, low-frequency operations would avoid possible alterations of the biological samples. The chip presents anyway a guard ring to shield environmental interferences.

The geometric and electric parameters of the planar microtransformers are summarized in Table 1. The inductance values have been calculated by using a modified Wheeler equation [13].

2.2. Device characterization

SEM images were obtained using a high-performance Schottky field emission LEO 1550 SEM Instrument operating at 5 kV in secondary-electron imaging mode.

The thermal-electrical characterization has been carried out by Karl Suss probe station with a thermal chuck (ModelPA200). The chuck temperature can be regulated from −40 °C to 160 °C. The electrical data have been acquired by using a Semiconductor Parameter Analyzer 4155C.

The experimental characterization has been perfomed in uniform temperatures conditions across the wafer thanks to the use of the heated chuck allowed the uniformity and distribution on wafer.

A Fluke thermocamera has been for thermal images acquisition.

ScreenMAG-Amine beads have been purchased by Chemicell GmbH. These beads are magnetic fluorescent silica particles with Hydrodynamic Diameter of 1.0 μm, core in Maghemite and matrix in non-porous Silica.

The device surface was treated with a specific chemical process to guarantee a good spot morphology and to create a coating suitable for further molecular grafting. In particular the device was cleaned by a plasma-O_2 process (Semitech equipment) for 10 min, with a plasma power of 100 W, and the cleaned surface properly silanized by an epoxy silane reagent by using a vapor phase process at 120 °C for 2 h in vacuum condition.

The deposition of microbeads on the surface of the secondary coils was carried out through a microspotter (Perkin Elmer piezo spotter) that allows the spotting of drops of 333 ± 43 pl. Microbeads solution at concentration of 12.5 mg/ml dispersed in sodium diphosphate 150 mM printing buffer (pH 9.2) containing an amount of 3×10^{-3}% of glycerol was employed. A number of spotted drops ranging from 3 to 60 corresponding to microbeads equivalent weight from 12 ng to 250 ng were deposed.

3. Results and discussions

Fig. 3 shows the device SEM cross-section in two different regions of the wafer. As respect to the geometrical dimensions reported in Table 1, we have observed a reduced track height and width for the primary coil: this feature has involved an enhancement in the primary coil resistance and inductance, respectively 1050 Ω and 3200 nH, and then a limitation in the maximum current flow, but not in the sensor working performances.

The comparison between the two images of Fig. 3 shows a good uniformity on wafer and a very good planarization process and thickness