BIOLOGICAL DETECTION LIMIT OF A GMR-BASED BIOCHIP FOR PATHOGENIC ANALYSIS

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Abstract

INESC-MN and its collaborators are pursuing the development of an integrated platform where the magnetic sensing unit, the biochemical interface (functionalized organic/inorganic surface), the macro/micro-fluidic system (sample preparation, cell concentrator, fluid transport), and the electronic data read-out and automation are combined in an easy-to-use and portable platform [1-3]. This contribution presents the latest achievements on the biochemical sector. A particular application is being targeted on the evaluation of microbiological quality of water for human consumption through the detection of waterborn pathogen microorganism. Two different strategies of biomolecular recognition events, involving DNA oligonucleotide sequences and antibodies as recognition agents, are under development for the quantitative determination of *Escherichia coli* and *Salmonella sp.* as model microorganisms in water samples. The biological detection limit of the device was assessed using 20mer single stranded DNA probes encoding to the genomic region of the 16S ribosomal sub-unit of *E. coli*.

The device consists in 24 U-shaped (2.5 x 80 μm^2) spin-valve sensors array of multi-layer structure Ta 15Å/NiFe 30Å/ CoFe 25Å/ Cu 21Å/ CoFe 25Å + oxid./ MnIr 80Å/ Ta 20Å/ TiW(N) 150 Å, fully passivated with 300 nm oxide layer. Additionally, a 200 Å gold thin-film was RF sputtered and gold pads of 43 x 13 μm^2 were defined precisely on top of sensor sites (Fig. 2). After a meticulous cleaning step, thiol-modified DNA oligos were directly attached to the gold surface and further used as biorecognition probes. Hybridized target molecules were labeled with 250 nm diameter magnetic particles (Micromod, Germany) through a biotin-streptavidin binding system. During the experiment 16 sensors were sequentially monitored in real-time applying 1 mA bias current through each sensor and recording an ac voltage change with a lock-in amplifier technique. An in-plane ac excitation field of 13.5 Oe rms at 31 Hz plus a dc field of 30 Oe were applied to magnetize the nanoparticles and to center the sensor transfer curve in the operating region.

Complementary target DNA solutions at different concentration, ranging from 1 μM down to 1 pM, were measured through the detection of nano-sized magnetic labels, originating average voltage signals of 500 μV down to 77 μV, respectively. The background noise associated to a measurement when no target DNA is present in the hybridization solution is 29 ± 13 μV. Moreover, in the presence of a non-complementary target (70% mismatch) at 1 μM the sensor detection signal is around 43 ± 30 μV (Fig. 1).

[1] Graham, D. L., Ferreira, H. A., Freitas, P. P., Magnetoresistive-based biosensors and biochips, *Trends Biotechnol* 2004**,** 22, 455-462.

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[3] Cardoso, F. A.; Germano, J.; Ferreira, R.; Cardoso, S.; Martins, V. C.; Freitas, P. P.; Piedade, M. S., Sousa, L., Detection of 130 nm magnetic particles by a portable electronic platform using spin valves and magnetic tunnel junctions sensors, *J. Appl. Phys.* 2007, in press.

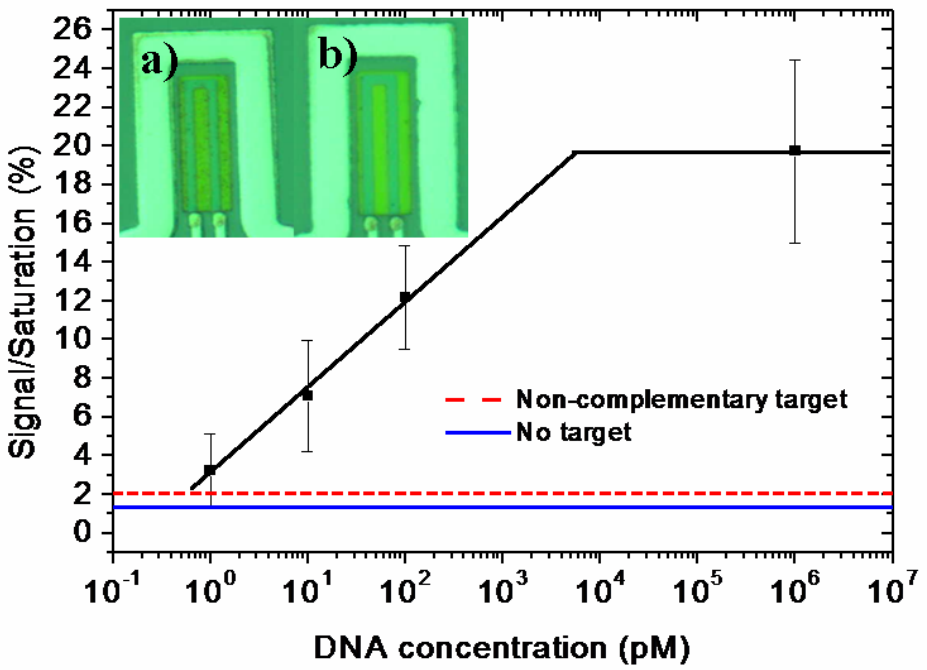

Fig. 1: Biological detection limit for single stranded DNA sequences encoding for the genomic region of the 16S ribosomal sub-unit of *E. coli*. Optical microscope picture at 800x magnification, from individual sensors corresponding to a) 1 μM target and b) no target assay.

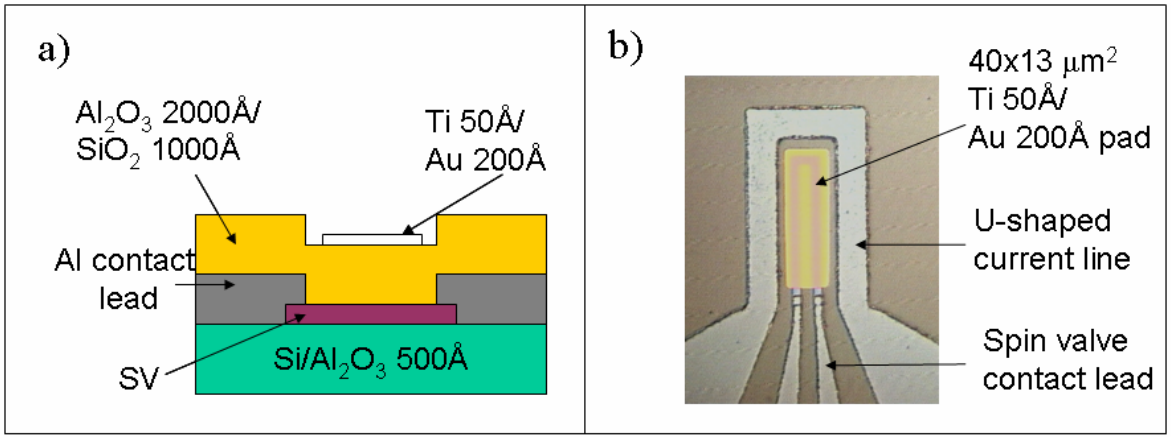

Fig. 2: Cross (a) and top view (b) of a spin valve chip sensing site showing the sensor, focusing current line, passivation layers and gold pad.