Magnetoresistive biochips

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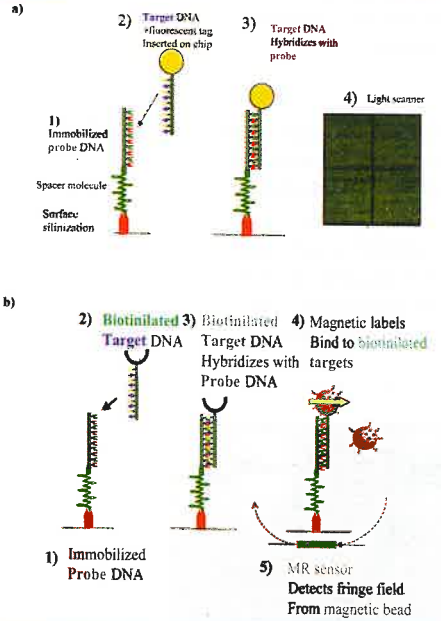
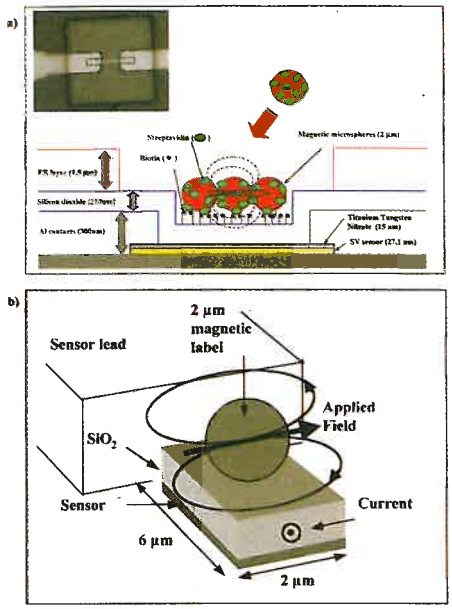
Detection of biomolecular recognition has been playing an ever important role when applied to DNA-DNA hybridisation (genetic disease diagnostic, mutation detection, gene expression quantification) and antibody-antigen interaction (micro-organism detection, biological warfare agent detection, etc...). A typical DNA biochip will consist of an array of probes (for example gene specific oligonucleotides that were immobilized onto the functionalised chip surface through microspotting), an hybridisation chamber (normally a microfluidic channel arrangement) together with an optional target arraying mechanism (electric fields for charged molecules such as DNA), the target biomolecules (eg, a complementary DNA strand to the immobilized DNA probe), the label (a fluorescent molecule that can be attached to the target), and a hybridization detection mechanism that can be either integrated on the chip or external to the chip (for instance, in the case of fluorophore labels, detection is done by an external laser-based fluorescence scanner) [1,2]. An hybridisation detection experiment (see Fig.1a) occurs through four phases, respectively 1) probe immobilization on chip surface, 2) target labelling, 3) target arraying, hybridization and washing, 4) detection. Requirements on the detection scheme depend on the particular biological assay.

For *gene expression* chips, where the relative amount of a certain gene must be quantified and compared between different patient samples, the detection scheme must lead not only to the knowledge of the presence of the particular gene (Yes or No) but also to a quantitative analysis (present in what percentage). For *Single Nucleotide Polymorphism* chips (SNP), where single DNA base pair changes are being identified, the simple (Yes or No) answer is sufficient. In both cases, the chip should be able to discriminate against false positives (non-specifically bound molecules). A recent example of this technology are DNA microarray chips fabricated by Nanogen [3], where charged target biomolecules are moved over immobilized probes. Electric fields are used for hybridization enhancement and stringency control.



▲ Fig. 1: a) Conventional hybridisation detection using fluorescent labels attached to biological targets, and an external laser-based fluorescent scanner for detection.
b) magnetoresistive-based hybridization detection using magnetic labels, and an integrated magnetoresistive sensor array for detection.



▲ Fig. 2: a) Cross-section of a MR-biochip, showing the spin valve sensor, the leads, the 2000Å thick SiO_2 functionalized passivation layer, the immobilized probes (in this case biotin), and the hybridized targets (streptavidin) coating the magnetic labels. The inset shows the top-view of the probe-pad. b) Detection geometry: a 150e in-plane field magnetizes the superparamagnetic labels, and in turn, these produce a transverse in-plane field in the spin valve).

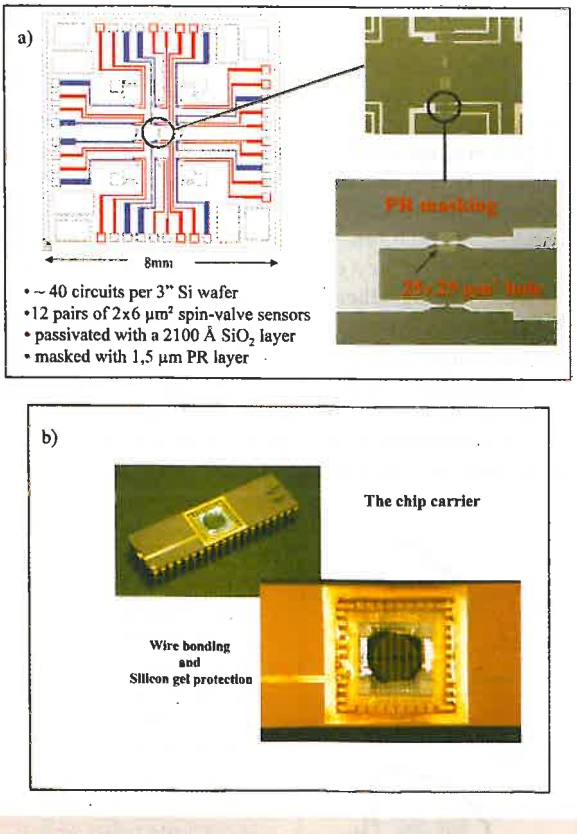
Detection is external to the chip, done by a laser-based fluorescence scanner. Fluorescence-based systems suffer from gradual loss of label fluorescent emission upon light excitation (photobleaching), and require careful background signal subtraction. Alternative approaches are being pursued in various labs to incorporate other labels (or no labels at all), in order to fully integrate the detection mechanism, aiming at a fully electronic and microfluidic portable and cheap apparatus, of widespread use.

MR-Biochips

Magnetoresistive-based biochips were first introduced in 1998 [4,5]. Fluorophore labels are replaced by magnetic labels (superparamagnetic particles), and detection is done using an integrated magnetic field sensor (GMR multilayer [6], spin valve [7], AMR ring [8], Hall effect cross [9]). The hybridization detection process is shown in Fig.1b. Since the magnetic labels used so far have been relatively large (from 100nm up to 2 μm in diameter) [10],and may hinder the hybridization process if previously attached to the targets, hybridization occurs between the immobilized probe and a biotinylated complementary target (no label, step 3). Streptavidin coated labels are added in a post-hybridization step (4), and detection is done in real-time. The use of magnetic labels allows the use of magnetic fields for stringency control, as well as for arraying (if previously attached to targets). Since magnetic material is usually not present in biomolecules, background signal subtraction is greatly simplified. Fig.2a) represents the cross-section of the MR-biochip at the sensor area.

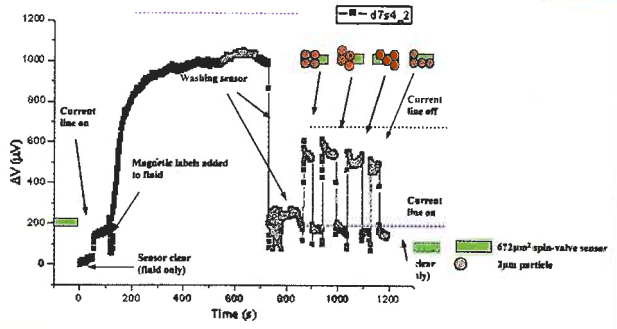
The MR sensor is passivated by a 0.2μm oxide or nitride layer (required to prevent sensor damage by salt solutions during immobilization, hybridization and washing).

The MR sensor directly measures the in-plane transverse field created by the magnetized label. For applied fields of 15 Oe, 2um Micromod particles (15% FeOx content) have a moment of 10^{-12} emu, creating a maximum transverse bead field of about 1 Oe on the sensor. Fig.2b) shows the detection geometry when using an in-plane field to magnetize the beads, and spin-valve sensors for detection. Depending on the biological assay, the sensor architecture must be designed for its particular application. For example consider μm size labels. If a linear response to the number of labels (up to few hundred) is required, as for example in a *gene expression* chip, the sensor can be made of a spin valve or GMR material in a meander configuration occupying most of the area under the probe pad [6,11]. The output is proportional to the area of the sensor excited by the particles fringe fields. For a spin valve sensor [12], the linear range can be easily tuned from 10 Oe to few hundred Oe either by reducing sensor height and increasing the demagnetising field, or implementing a longitudinal exchange bias field H_{bias} onto the free layer. The dynamic range for these sensors allows measurements from few to few hundred particles. If on the other hand, a *SNP* chip is envisaged, the spin valve sensor dimension can be tuned to that of the particle, and single labels with moments as low 10^{-14} emu should be detected. This minimum detectable moment (field) is limited by the sensor noise [10], and measuring electronics setup (DC vs lock-in detection). MR sensors are normally used in a Wheatstone bridge configuration in order to minimize thermal offsets and to null bridge response in the absence of particles. Fig.3a) shows a typical SNP spin valve sensor biochip , emphasizing the differential detection scheme, while Fig.3b) shows the picture of a packaged device,



▲ Fig. 3: a) Schematics of one of the MR biochips, using differential detection, with one active sensor and one reference sensor as two arms of a Wheatstone bridge configuration. b) Packaged MR biochips ready for biological assaying.

features



▲ **Fig. 4:** Bead detection using spin valve sensors. After sensor saturation (1mV) , beads are washed away, and repeatidly brought over the sensor. Number of beads is checked by optical microscopy. The sensor signal reproduces clearly the bead immobilization onto and release from the sensor.

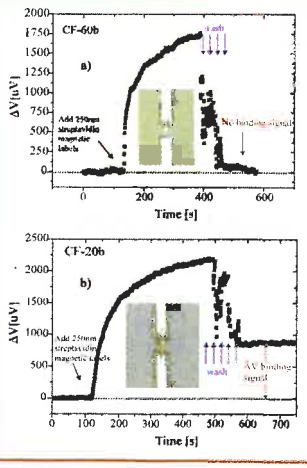
ready for biological assaying.

Fig.4 Shows detection signals for few (1-4) 2μm particles over a 6×2μm² spin valve sensor with an MR signal of 8%. For sensor linearization, the sense current is set at 8mA.

An in-plane transverse field of 15Oe is used to magnetize the superparamagnetic beads. A simple DC method was used to measure the sensor output(10μV noise level). For this experiment, the labels were controllably moved across the sensor using field gradients created by auxiliary current lines. Typical signals for single beads are of the order of 150uV/bead.

DNA-cDNA hybridization detection: cystic fibrosis CFTR gene recognition

As an example of DNA-cDNA hybridization recognition using the MR biochip a cystic fibrosis specific 50mer (50 base pairs) oligonucleotide corresponding to a particular strand of the CFTR gene was immobilized onto the 2000 Å thick, sputtered SiO_2 probe pad, covering the MR sensor area. The goal is to detect whether a complementary target will bind or not. Prior to probe immobilization, SiO_2 functionalization was achieved by surface silinization followed by a cross linker placement. Hybridization was attempted using either complementary or non-complementary biotinylated DNA target sequences. After hybridization and washing to remove non-specifically bound DNA targets, streptavidin functionalized magnetic labels are introduced in solution. These attach to the bound, biotinilated complementary DNA targets. Real time measurement of the sensor output during multiple label injection and washing cycles show (see Fig.5a) that where



◀ **Fig. 5:** a) Detection of DNA recognition using streptavidin coated 250nm labels, and a biotinylated complementary target. After washing to eliminate non-specifically bound molecules, a strong remnant signal remains, from the bound complementary targets. b) When a non-complementary target is used, after washing, no remnant signal is observed.

hybridization has occurred, MR sensor remnant signals of 1mV are observed, corresponding to about 100 nanoparticles (250nm) bound. Direct optical analysis confirms the MR signal results. When a non-complementary DNA strand is used (Fig.5b), no remnant signal is observed.

Conclusion

MR technology is being successfully applied to biomolecular recognition in different contexts. Advances are required in the development of biocompatible magnetic labels with higher moments (eg, pure Co, Fe, NiFe particles), and nanometric dimensions (< 100nm) providing clustering can be avoided and biocompatibility assured. Developments in the sensing technology are required to allow measurements of single nm-sized labels with moments at the 10^{-15} emu level. Developments are required in the use of magnetic and electric fields for arraying, hybridization enhancement, and force discrimination of non-specifically bound biomolecules. MR technology has shown the potential for single molecule process detection, a target not usually within the reach of most of the competing technologies.

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