

## **MAGNETORESISTIVE DETECTION OF FLOWING AND IMMOBILIZED ASSAY LABELS**

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### **ABSTRACT**

Magnetic nanoparticles are used in many biochemical assays as labels for concentration, manipulation and, more recently, detection. Typically one attaches the magnetic particles to the biochemical species of interest (target) using a chemically specific binding interaction. Once bound, the labels enable the manipulation of the target species through the application of magnetic forces. Spintronic sensors, specifically Giant Magnetoresistive (GMR) and Spin Dependent Tunneling (SDT), sensors have been developed to detect and quantify labels in two main formats: flowing in a microfluidic channel, and immobilized labels on a chip surface. Designs and data for these two types of assays will be presented.

## INTRODUCTION

Spintronics Technology Giant MagnetoResistance (GMR) and Spin Dependent Tunneling (SDT) technology (spintronics) is typically implemented in a fabrication process that looks very much like that at an integrated circuit IC foundry. Spintronic devices are built on silicon wafers using vacuum deposition machines, and are patterned using standard IC lithographic tools. The details of a given spintronic design depend upon what application they are used for, but they all end up being thin film resistors with thicknesses on the order of a few nm, and widths on the order of 1  $\mu\text{m}$ . Their resistance depends on the relative magnetization orientations of ferromagnetic films within the multilayer spintronic stack. The resistance is lower when the magnetizations are parallel, and higher when the magnetizations are antiparallel. The magnetic films can be designed to have two stable orientations for the “free layer” or allow the free layer magnetization to rotate smoothly over the whole 360 degrees. The former case is typically used in a memory application where information is stored in the magnetization orientation. The latter case is typical of a magnetic field sensor, where an externally applied magnetic field causes the magnetization to rotate smoothly through some angle. Spintronic biosensors use the linear sensing type of spintronic devices.

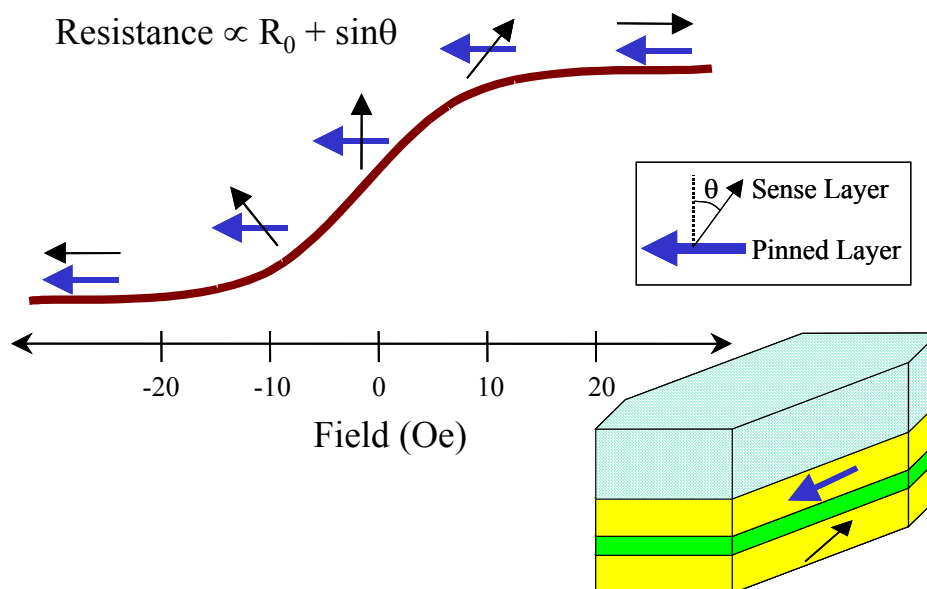


Figure 1. The resistance vs. applied field response of a linear spintronic sensor of the “spin valve” variety. The thick arrows indicate the magnetization orientation of the “pinned layer” which is designed to remain fixed along the axis of sensitivity. The thin arrows indicate the magnetization orientation of the “sense layer” or “free layer.” The sense layer rotates smoothly when an external field is applied.

### Spintronics + magnetic nanoparticles + microfluidics = Biosensors and Lab-on-a-Chip

Magnetic sensors are used in a wide range of biomedical and biochemical applications, including Magnetic Resonance Imaging (MRI) and pacemakers. A relatively new kind of application is the “Lab-on-a-Chip” (LOC) devices, where sensors, sample handling, and data gathering and analysis take place in an integrated system whose size is decreasing rapidly towards a cubic cm. In this arena, the value of spintronic sensors is magnified because it is easy to make it small and in a chip form. This is in contrast to many assays relying on optical means to obtain data. The optics are not easily integrable into a chip format even if the rest of the LOC is.

For spintronic sensors to be useful in LOCs, the assay being performed has to have a magnetically readable signal. This is usually generated by attaching magnetizable magnetic nanoparticles in ways that provide a magnetic signature that is in proportion to the quantity of biomolecules present in the sample. Obtaining quantitative results from LOCs requires that care be taken with the sample handling and preparation, because the effective sample volume that it can process and measure is very small. On the other hand, the ability to handle very small volumes leads to very high precision in some assays. Microfluidics is the technology used to design and create this precision fluid handling for LOC devices. Of the three technologies, spintronic sensors, magnetic nanoparticles, and microfluidics, the latter is the least developed. The present state of the art is such that there are virtually no consumer-available microfluidic products.

This paper discusses the state of the art for spintronic detectors in a microfluidics format from three angles, manufacturability, commercializability, and technical performance. Some laboratory examples of magnetic bioassays are shown in the applications section. Presently, magnetic bioassays are moving from a “research” phase to a “development” phase. Estimation of commencement of the “product” phase is provided in the commercializability section.

## MANUFACTURABILITY

Manufacturing devices using a wafer-scale (handle 1000s of sensors at a time on each wafer) process is very attractive from a pricing standpoint if sufficient yields can be achieved. Because the cost to process a wafer is roughly constant, there is always a push to decrease the individual sensor die size: small dice are cheap dice. Imposing the semiconductor manufacturing model on biosensors and LOCs presents several technical challenges in the areas of biochemistry and microfluidics integration. Some so-called silicon-based microfluidics technologies ignore basic manufacturing cost realities, and ultimately deliver impractical devices. NVE’s approach has been to incorporate Lab-on-a-Chip technology into manufacturable process flows.

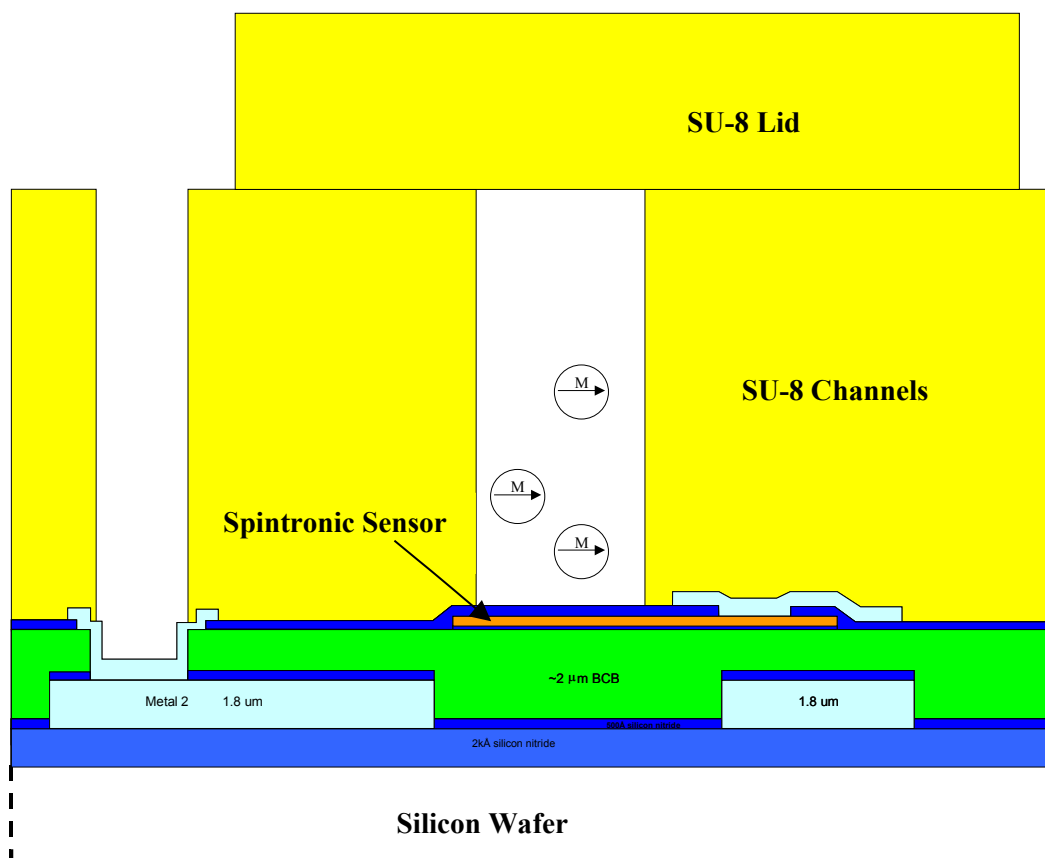


Figure 2. Spintronics + microfluidics fabrication process cross section. The spintronic sensor is a thin layer that sits directly beneath fluid channels. The channels are aligned and formed on the spintronics wafer using SU-8 or other polymers. Magnetic nanolabels are shown in the fluid channel.

**Fluids** Combining fluids and circuits is normally considered to be hazardous, but must now be done by design. And biochemical assay fabrication methods are not always compatible with wafer handling processes.

**Size** While the natural size of a successful silicon circuit product may be 800  $\mu\text{m}$  on a side, a typical biochemical assay specimen may be much larger than this. A  $\text{cm}^3$  is not uncommon, and the various sample handling procedures must be performed in sufficiently sized chambers, with applied pressures and valves. In the near term, it is unlikely that the Lab part of LOCs will be small enough to fit on a real circuit chip. Plastic molding is the most economical way to fabricate the fluidic interfaces. The manufacturing challenge is, then, to efficiently combine silicon-based chips with plastic cartridges.

**Materials** There are many materials that provide electrical, fluid, and chemical isolation in a chip format. Glass and SiO dominated early work in microfluidics because they are familiar to biochemists and silicon foundries. Getting good encapsulation of etchable glass layers while maintaining a temperature profile that other aspects of the wafer devices could handle has proven impractical. Most LOC fabrication development has moved towards polymers and plastics as interface layers to molded plastic fluidics cartridges.

## APPLICATIONS

Most potential biosensor applications using magnetic nanolabels are based on the desire to quantify the number or concentration of a given target biomolecule, cell, or bacteria, in a given sample volume. These targets, by themselves, are not readily detected in a convenient way. So a common strategy is to attach a "label" which is readily detected. Then the biosensor function is to count labels, while making sure that the labels being counted actually correspond to known amount of target material. Labeling can be accomplished in a variety of ways. In Fig. 3 below is described, in cartoon form, one way of labeling for a DNA type assay. In this case, the magnetic nanolabels are *immobilized* on the chip surface and then counted. A top view of this type is shown in Fig. 4. Another technique, which is at an earlier stage of development, is detection of *flowing* magnetic nanolabels. This type of assay is depicted in Fig. 5 below.

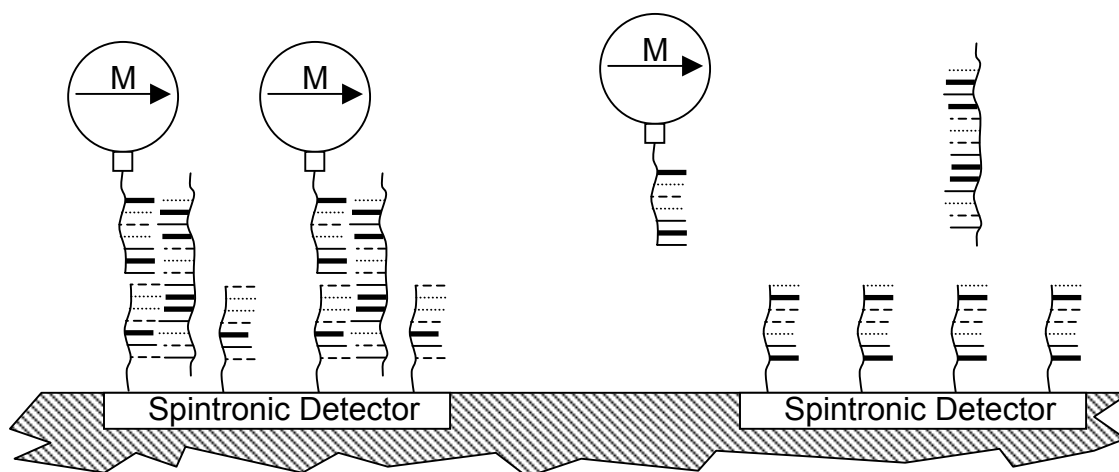


Figure 3. Biochemical assay labeled with magnetic nanoparticles. The spintronic detectors are embedded in the biochip surface. Specific sequences of single stranded DNA (chip probe), matching part of the intended single stranded target DNA, is attached to the chip surface immediately above the spintronic detector. Sample target DNA, present in the fluid surrounding the spintronic detectors, hybridizes with the chip probe DNA. Then label probe DNA, bound to some nanomagnetic labels, hybridizes to the exposed portion of the target DNA. In this way, magnetic labels are immobilized on the chip surface in a selective way. The spintronic detector is then used to determine the number of nanomagnetic labels at each site.

**Spintronic Assay Performance** can be measured in a few ways. One can cite the minimum number of labels required to generate a detectable signal, the dynamic range of labels that is detectable, or the concentration in sample solution of the target analyte. Spintronic detectors have generated favorable results in all three ways of measuring. Single magnetic label detection has been demonstrated, both for spherical particles [NRL BARC] and rod-shaped magnetic nanowires [JHU]. Detectors that are capable of detecting a single particle tend to be fairly small, say with length and width on the order of the particle diameter. Most real life assays, however, do not benefit from single particle detection limits because they need to see a much greater quantity of biochemical sample to obtain valid experimental results. A detector with a larger surface area, then, is able to acquire a better statistical survey of the target analyte concentration in the sample solution. In this kind of detector, the dynamic range is a better way to quantify precision. The useful dynamic range of detectable particles is about three logs (e.g. a detector can count from 10 to 10,000 labels, which is three orders of magnitude). One exciting aspect of spintronic detectors is that, by dividing a large sensor area into many small sensors, the dynamic range can be greatly extended. This multi-sensor approach takes advantage of the intrinsic compatibility of spintronic sensors with integrated switching circuitry. Integrated switching of spintronic detectors is in the early stages of development. The best results using spintronic detectors from a sample concentration standpoint is about 10 femtoMolar [Rife, et. al Naval Research Lab.].

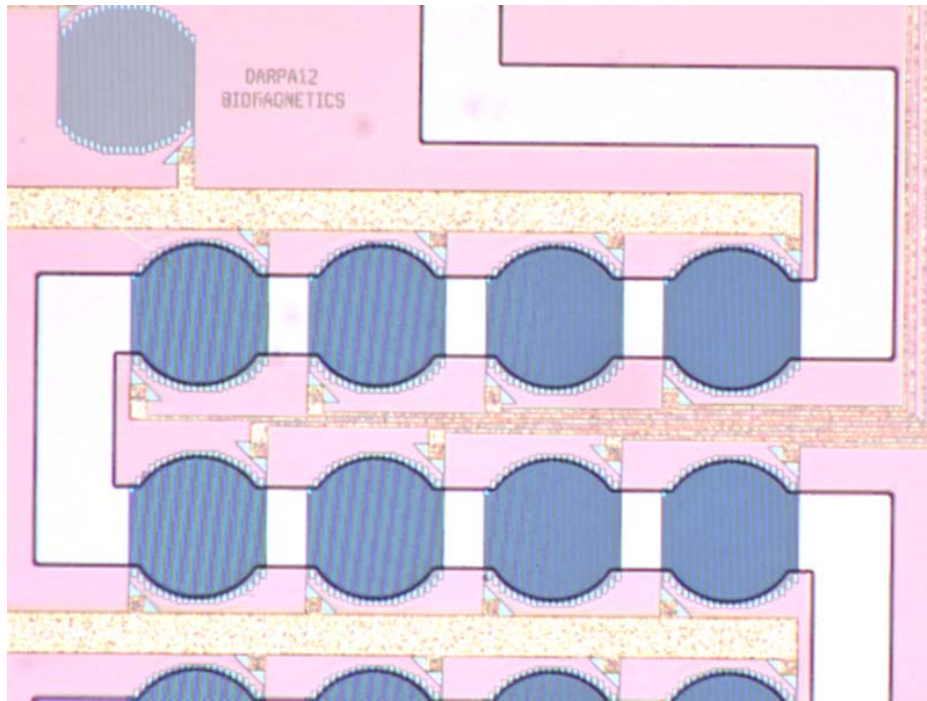


Figure 4. Photograph of a GMR sensor array. This is an array of detectors with a microfluidic channel passing over the top in a sequential manner. The full chip has a 4 x 5 array (20 sensors) with two reference sensors. One of the references is visible at the upper left. Each of the round GMR sensing areas is about 200 microns in diameter, and the channel is about 100 microns deep.

The detection of magnetic nanolabels in a flow stream is still in the early research stages. Fronts of nanoparticles have been detected moving over a sensor [Freitas, et. al. INESC-Portugal], as have plugs of ferrofluid [Pekas, et. al., Iowa State Univ.]. Neither of these tools is yet fully adapted to biochemical applications.

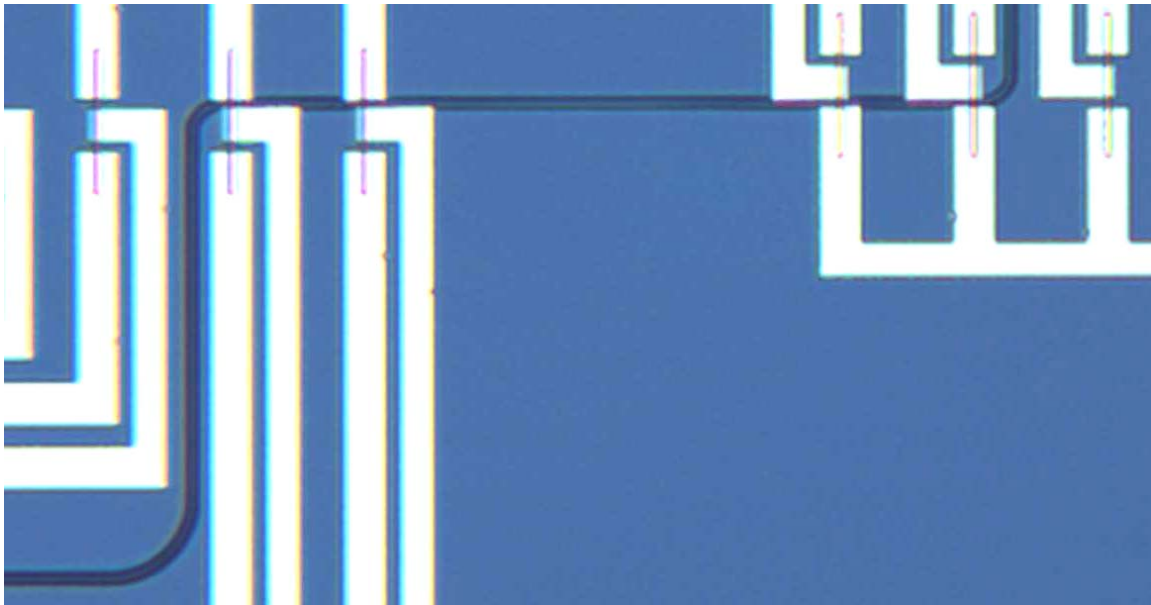


Figure 5. Picture of channel for flow detection. The channel enters the picture at the lower left and leaves at the upper right. It passes over two GMR sensors on the left half of the picture, and two more on the right half. The channel is about 10 microns wide.

Nanomagnetic labels are available in a wide range of styles, and from about 20 vendors [see <http://www.magneticmicrosphere.com/supply.htm> for a list]. Their size ranges from 10 nm to 10,000 nm. Their magnetic properties can range from solidly ferromagnetic to small percentages of magnetic material. Their biochemical function depends on how they are coated. One can obtain particles with biotin or streptavidin coatings, and many antibodies. Many companies will perform custom “functionalization” of their particles for unique applications.

### **COMMERCIALIZATION PATH**

Because spintronic biosensors are based on a tiny low-cost detectors, they appear to have intrinsic advantages in high-volume disposable product applications. Their ruggedness in comparison with other technologies is also a benefit, particularly for military uses (which has substantially driven development to date). High volume applications, especially consumer driven areas like pregnancy tests, are attractive targets for commercialization. Though presently limited in variety, it is expected that genomics and proteomics development will lead to a plethora of consumer assay applications providing the cost structure is right.

The first spintronic biosensor products will be small sensor arrays ( $\leq 64$  sites) for which existing sensitivity is sufficient. The fluidics will likely be performed with external handling modules. At least one company [Seahawk.com] has indicated their intentions to develop spintronic based assays for the veterinary market. Later products will have greater sophistication in terms of number of sensors (could be millions for a large DNA assay), and/or the degree of fluidics integration (incorporating pumps, valves, mixers, on a fluidics card/chip).

There are several competing technologies in the biosensor area. Some of them are already well established in research and development markets. Some examples are those based on optical, surface plasmon resonance (SPR), electrochemical, and amperometric detectors. Spintronic detectors offer potential technical advantages over these other technologies for many anticipated applications. Generally, they provide a high level of detector performance on a platform that supports a high degree of circuit and “imaging” precision and sophistication at relatively low cost.

### **CONCLUSION**

Magnetics-based biosensor chips offer unique advantages in sensitivity, manufacturability, and ruggedness. The leading edge of this technology is moving from research into development, and some products will likely appear in the 1 – 2 year time frame. The ultimate range of applicability could be quite large as LOCs and related microfluidics technologies become fully accepted in the marketplace.

### **ACKNOWLEDGEMENTS**

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