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Evolution of a Magnetic-Based Biomolecular Detection System

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***Abstract*—Amongst the plethora of affinity biosensor systems based on biomolecular recognition and labeling assays, magnetic labeling and detection has emerged as a promising approach. Magnetic labels can be detected by a wide range of non-invasive methods, are physically and chemically stable, relatively inexpensive to produce, and can be easily made biocompatible. Over a decade ago, the U. S. Naval Research Laboratory pioneered the use of giant magnetoresistive (GMR) sensors to detect biomolecules labeled with paramagnetic microbeads. Since then, our various investigations and engineering efforts have resulted in significant improvements in both the magnetoelectronic instrumentation and the assays associated with these magnetic labels. This paper and subsequent presentation provides a synopsis of the development of our technology which has evolved into a highly sensitive detection method.**

I. Introduction

SENSOR systems based on biomolecular recognition and labeling assays are under intense development for many applications, including biodefense [1], medical diagnostics [2], food safety, and environmental monitoring [3], [4]. The most important component of affinity biosensor systems is the assay. It is the fundamental piece, arguably of greater importance than instrumentation performance, because it defines the ultimate sensitivity of a particular biosensing technology. A general goal of labeled bioassays is to combine the innate sensitivity and specificity present in nature with an easily detectable taggant. As such, the most common approach to biosensing is the implementation of solid-phase binding assays whereby a chemical label that produces an externally observable signal is attached to a biomolecular target tethered to a solid substrate. Traditionally, this attachment is accomplished using biomolecular recognition between the target molecule and a specific receptor (e.g. an antibody) that incorporates a label, or "reporter," such as a fluorophore, radioisotope, enzyme, nanoparticle, or electrochemically active species. Then, to detect these reporter labels, a number of transduction mechanisms have been devised, including optical, electrical, electrochemical, thermal, and piezoelectrical, all of which are covered eloquently in review articles [5], [6].

Out of the wide choice of labels that can be applied to common assay schemes, magnetic particles offer some unique advantages [7]. Notably, there is no significant magnetic background present in most samples of interest, thereby enabling detection and magnetic manipulation, both *in vitro* and *in vivo*, without disturbing the biological matrix. A prime requirement for use as labels is that the beads must be paramagnetic or non-remanent to avoid clustering caused by residual magnetic moment in the absence of magnetic fields. Microscale paramagnetic beads have been extensively developed primarily for cell and protein separation, and are available from a number of commercial sources. There is a growing interest in magnetic labels because of their ever increasing use in magnetic sensing strategies, their physical and chemical stability, their inexpensive methods of production, their environmental safety, and their biocompatibility.

II. Magnetoelectronic Detection Technology

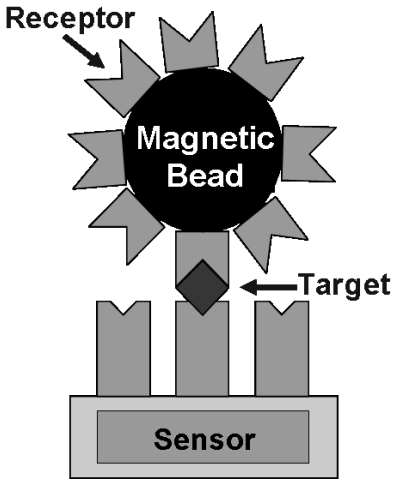

Fig. 1. Cartoon of a standard "sandwich" type assay utilizing biofunctionalized magnetic microbeads as labels. Through natural molecular recognition between receptors and ligands ("target"), the magnetic bead becomes tethered to the sensor surface and detected.

As applied in our approach, a bioassay is performed which ultimately results in a paramagnetic microbead being tethered to a substrate surface through a specific receptor/ligand (or "target") interaction (Fig. 1). Full details of this assay will be described below. Once tethered, there are GMR sensors embedded in the silicon substrate that

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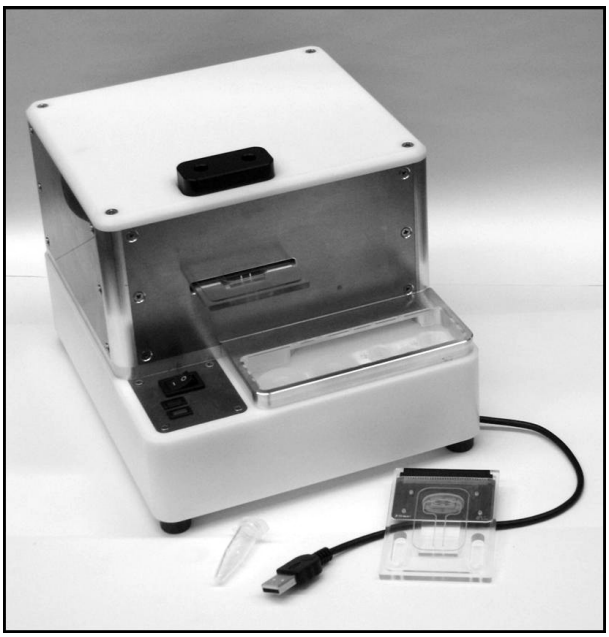

Fig. 2. The latest version of NRL's shoebox sized compact Bead Array Sensor System (*cBASS*®). It is a fully automated device with integrated signal processing electronics and microfluidic actuation system. Assays are performed on the BARC® chip mounted in a microfluidics cartridge.

"picks-up" the local magnetic fields that emanate from the bound microbeads.

In a simplified description of a GMR device, the resistance of two thin anti-ferromagnetically exchange-coupled layers separated by a thin nonmagnetic conducting layer can be altered by changing the moments of the ferromagnetic layers from antiparallel to parallel. This change decreases the spin-dependent interfacial scattering of charge carriers resulting in a decrease in the resistance of the GMR material. The sensitivity of magnetoresistive (*MR*) materials is expressed as the change in resistance divided by the minimum resistance, or

$$MR_{ratio} = \frac{\Delta R}{R} = \frac{R - R_{Hsat}}{R_{Hsat}} \times 100\%, \qquad (1)$$

where R_{Hsat} is the minimum resistance in a saturation field [7]. Ratio comparisons are commonly reported for measurements taken at room temperature. Simple multilayer GMR devices exhibit a magnetoresistance ratio between 4 and 9% [8].

Several important devices have utilized the phenomenon, most notably magnetic recording (read heads in hard drives) and magnetic non-volatile memory. In 1998, Baselt et al. described a new concept in biological labeling and magnetic sensor detection based on GMR sensors [9]. They described a semiconductor-based multilayer GMR sensor chip, which came to be known as the Bead ARray Counter (BARC®) chip that detects local in-plane magnetic fields produced by paramagnetic microbeads immobilized directly above the sensor surface during binding assays. As our investigation progressed, the idiosyncrasies of paramagnetic microbead detection by GMR were revealed. Several engineering solutions were developed to address microfluidic handling, detection electronics, and assay issues. The result is our fully automated biosensor device called the compact Bead Array Sensor System (*cBASS*®) (Fig. 2) [10]. Among other important features, it now utilizes the BARC®-III chip with 64, 200 μm diameter sensing zones, a low-power electromagnet design, an onboard fully automated fluid actuation system, a quick assembly assay cartridge with integrated microfluidic cell, and fast data exchange via USB with a controlling computer. The current version of this 6 mm x 12 mm BARC® chip has reached a lower limit of detection equivalent to the signal of between 3–10 beads [11]. BARC® and other magnetic label detection technologies have recently been compared in an extensive review [7]. When magnetic particle sensitivity, sensor area per detectible particle, and technological simplicity is considered, BARC® exhibits the best overall characteristics for magnetic biosensing.

III. Assays: Biomolecular Labeling with Paramagnetic Microbeads

With its ability to detect low bead numbers, the overall detection sensitivity of *cBASS*® is in fact not limited by the electronics, but by the sensitivity of the associated assay. Therefore, significant emphasis is placed on the development of our assays such that they not only function in various matrices (e.g. blood, wastewater, food, etc.) [12], [13], but are able to deal with a common scourge of bioassays: non-specific binding. The assays used with *cBASS*® are the typical "sandwich" style and proceeds as follows:

1. Receptor molecules specific for the target biomolecules are attached to the surface of a solid substrate. Often arrays of different probe spots are used to simultaneously detect multiple targets.
2. When target molecules are present in a sample solution, they are captured at the surface.
3. Paramagnetic particles coated with a second set of receptor molecules for the target are introduced, labeling the previously captured targets.
4. The label particles are detected by a magnetic sensor.

Sandwich assays are commonly performed using antibody-antigen pairs or complementary DNA strands, and often use the strongly binding biomolecular combination of streptavidin and biotin to attach the receptor molecules to the bead or substrate surface. One of the most important advantages of using a physical label such as microbeads, is that given the right bead size, knowledge of the fluidic channel dimensions, and the forces which bind complementary biomolecules (e.g. approx. 60–250 pN for immunochemical complex, approx. 800–1200 pN for DNA duplex), one can perform what we call Fluidic Force Discrimination (FFD) to actively remove non-specifically bound (approx. 0.1–10 pN) microbeads under a controlled

fluidic flow [13], [14]. This has allowed us to achieve nucleic acid assays with femtomolar (fM) sensitivities [13] and immunoassays with attomolar (aM) sensitivities [15].

IV. *cBASS*® On-Chip Assay

An immunoassay for the protein toxin Ricin (RCA) is a typical test that can be run on a BARC® chip [13]. The detection of 10 ng/mL of RCA in bovine serum is described in this example. Antibody probes for RCA were spotted on selected GMR sensors, followed by target capture, and finally paramagnetic microbead capture via conjugated secondary antibody. As a negative control, selected sensors have also been arrayed with probes for another protein toxin *Staphylococcal enterotoxin B* (SEB). Once magneto-electronic detection of the paramagnetic labels is completed, the BARC® chip can be observed under a microscope to optically count the tethered beads on the GMR sensors for experimental verification (Fig. 3). The average signal per bead is based solely on magnetoelectronic detection and is therefore unaffected by the sample matrix and independent of the assay method.

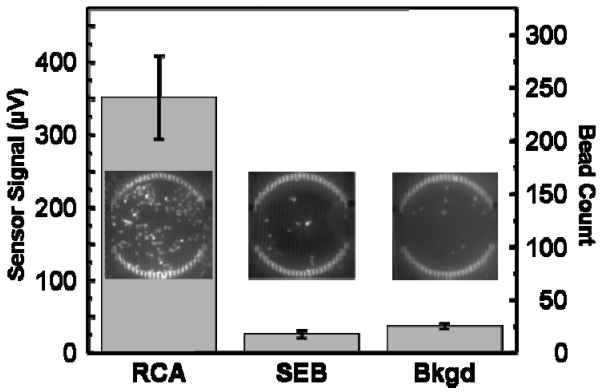

Fig. 3. Graph showing the average GMR sensor response for the detection of 10 ng/mL RCA in serum. The left axis indicates the average voltage measured per sensor, which is linear in bead count up to ~1000 beads. The right axis indicates the average optical bead count per sensor. The relationship between magnetic signal and optical bead count is ~1.6 µV/bead. Representative micrographs of a sensor from each capture zone are provided to show that the beads can be individually resolved and easily counted.

V. Conclusion

In an ever growing body of biosensing work describing techniques capable of critically analyzing minute quantities of biological samples, paramagnetic labeling offers unique advantages in false positive reduction, sensitive detection at aM levels, the ability to operate with complex matrices, and simple "sandwich" assay protocols that require no chemistries other than molecular recognition. In conjunction with magnetoelectronic detection using arrays of GMR sensors, compact system possibilities, such as that exemplified by NRL's shoebox sized *cBASS*®, are possible.

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