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Magnetoresistive sensor for real-time single nucleotide polymorphism genotyping

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ABSTRACT

We demonstrate a magnetoresistive sensor platform that allows for the real-time detection of point mutations in DNA targets. Specifically, we detect point mutations at two sites in the human beta globin gene. For DNA detection, the present sensor technology has a detection limit of about 160 pM and a dynamic range of about two orders of magnitude. The sensors are based on a new geometry for biological sensing that detects the difference between the amount of beads bound to a sensing pad and a local integrated negative reference pad. The magnetic beads are magnetised by the magnetic field arising from the sensor bias current such that no external magnetic fields are needed. The sensors are integrated in a microfluidic system with temperature control. The local negative reference integrated in the sensor geometry efficiently compensates for sensor offsets, external magnetic fields and a uniform background of magnetic beads, which enables real-time quantification of the specific binding of magnetic beads to the sensor surface under varying experimental conditions.

1. Introduction

Genotyping has wide application in diagnostics of genetic diseases, cancers, and viral and bacterial infections. There is an ever-increasing need for rapid, specific and sensitive methods to detect particular DNA sequences in a sample. Several diseases are related to alternative forms (alleles) of a given gene. For cancer and genetic diagnostics, the assays must be able to distinguish DNA species differing in only one base to perform so called single nucleotide polymorphism (SNP) genotyping. In some cases the SNP is known and specific probes against that particular SNP can be designed. In other cases, the SNP is unknown and must be screened for. Allele specific hybridisation is a rapid method for SNP genotyping that employs probes specific for the wild type (WT) and mutant type (MT) sequences. A simple hybridisation and dehybridisation reaction, most often with an end-point fluorescence readout, can then be used to obtain specificity. Multiplexing allele specific reactions is a substantial optimisation process and sequence dependent variation around each respective SNP makes it difficult to obtain fully optimised SNP calling at a given assay condition (Poulsen et al., 2011). The efficacy of SNP calling can be increased by treating the hybridisation at different stringencies by varying the salt concentration or the temperature (Howell et al., 1999; Poulsen et al., 2011; Petersen et al., 2008, 2009). The SNP calling can be performed after a given time point or in real time (Howell et al., 1999; Poulsen et al., 2011; Petersen et al., 2008, 2009). Studying real-time dehybridisation is preferable since far more conditions can be investigated as compared to post washing analysis. However, real-time fluorescence monitoring is hampered by high background signals from the fluorochromes used to detect the hybrids. Real-time investigation of hybridisation and dehybridisation can also be used to obtain high resolution melting curves for screening of unknown mutations in a sample (Er and Chang, (2012)). Such analyses are mostly performed in solution phase using intercalating fluorescent dyes. A corresponding analysis is possible using DNA microarrays (Nørholm et al., 2004), but it requires specialised and bulky instrumentation, and it is still hampered by high background signals from fluorochromes present in the sample.

Biosensors with a readout based on the binding of magnetic beads to the surface of a magnetoresistive field sensor based on the giant magnetoresistance (GMR) or tunnelling magnetoresistance effects have been proposed for the detection of DNA or proteins by a number of authors and are reviewed by Freitas et al. (2007), Tamanaha et al. (2008) and Wang and Li (2008). Common for most of these approaches is that the magnetic beads are excited by an oscillating applied magnetic field resulting in a signal detectable by the magnetic field sensor. The studies have focused on the end-point detection by comparing the signal level after a washing step to that before the sample was introduced. Recently, Gaster et al. (2009) demonstrated the first real-time measurements of the magnetic bead-binding to a GMR sensor surface due to protein interactions. These measurements were

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carried out in a background of magnetic beads and thus the need for washing was eliminated. Although the magnetoresistive sensing technology is promising, real-time measurements of the binding due to specific biological interactions have been limited to protein interactions.

In the present work, we demonstrate a novel magnetoresistive-based sensor architecture that allows for real-time detection of microarray-based DNA hybridisation and dehybridisation without the issues of washing steps and measurement of the background signal.

Previously, we have presented the design of magnetic field sensors in a planar Hall effect cross-geometry (Ejsing et al., 2004) and a Wheatstone bridge geometry based on the anisotropic magnetoresistance effect (Henriksen et al., 2010). The particular Wheatstone bridge configuration studied by Henriksen et al. (2010) gave the same response as the planar Hall effect sensor cross geometry except for a geometrical amplification factor and was hence named planar Hall effect bridge (PHEB) geometry. It has been demonstrated that sensors based on this geometry are well suited for dynamic measurements on magnetic beads in a liquid volume near the sensor surface using only the magnetic field from the sensor bias current as excitation (Østerberg et al., 2013a). Here, we present a new Wheatstone bridge sensor geometry, which integrates a local negative reference. We show that this enables the real-time quantification of beads that are *specifically* bound to the sensor surface due to biomolecular recognition, even in a background of magnetic beads in suspension and when the environmental conditions of the sensor are changed. Subsequently, we evaluate the sensitivity of the present sensors when used for DNA analysis and demonstrate their applicability for SNP detection.

2. Material and methods

2.1. Sensor designs

The PHEB sensors are based on the anisotropic magnetoresistance of a thin film of permalloy ($Ni_{80}Fe_{20}$) of the Wheatstone bridge geometry shown in Fig. 1a, which is exchange-pinned along the x-direction using an antiferromagnetic layer (Henriksen et al. (2010)). When biased with an AC voltage $V_x = \sqrt{2}\, V_{RMS}\, \sin(2\pi f t)$, a magnetic field H_y acting on the sensor area along the y-axis gives rise to a sensor voltage output along the y-axis, which can be measured by lock-in detection in the first harmonic in-phase signal. For small fields, the response is linear and given by $V_1' = (V_{RMS}/R) S_0 H_y$, where R is the bridge resistance and $S_0 (< 0)$ is the low-field sensitivity (see Supplementary Material). The current running in the sensor also generates a small inhomogeneous magnetic field in the proximity of the sensor surface (Hansen et al., 2010; Østerberg et al., 2013b). This self-field is used to magnetise the magnetic beads near the surface allowing for their detection with no need for external magnetic fields. The signal from magnetic beads over the sensor surface can be measured by lock-in detection in the second harmonic out-of-phase signal (Østerberg et al., 2013a), which can be written as

$$V_2'' = -\frac{1}{4} S_0 \left(\frac{V_{RMS}}{R}\right)^2 (\gamma_0 + \gamma_1) \tag{1}$$

A detailed derivation of this expression is given in the Supplementary Material. Here, γ_0 is a constant describing a sensor self-biasing due to current shunting in other layers than the magnetoresistive layer and γ_1 is a parameter accounting for the signal due to magnetic beads, which depends on the sensor width, the bead diameter, the bead magnetic susceptibility and the bead number density distribution in the volume over the sensor (including both beads on the sensor surface and in the volume over the sensor) (Hansen et al., 2010). The value of γ_1 is zero in the absence of magnetic beads and positive in the presence of magnetic beads.

Here, we propose a new sensor geometry termed differential PHEB (dPHEB) with identical top and bottom halves of the bridge as illustrated in Fig. 1b. This symmetry makes the sensor nominally insensitive to uniform magnetic fields. Moreover, since the self-field acts identically on the top and bottom parts, the second harmonic out-of-phase signal depends *only* on differences in the amount of beads on and over the top and bottom halves of the bridge, respectively. The second harmonic out-of-phase signal of the dPHEB sensor can be expressed as

$$V_2'' = -\frac{1}{8} S_0 \left(\frac{V_{RMS}}{R}\right)^2 (\gamma_{top} - \gamma_{bottom}) \tag{2}$$

where S_0 is the low-field sensitivity of a PHEB sensor with identical dimensions and γ_{top} and γ_{bottom} depend on the amount of beads on and over the top and bottom halves of the bridge. If the bead concentration in the liquid over the sensor surface is the same for the two sensor halves, only a difference in the amount of surface-bound magnetic beads is anticipated to give rise to a difference between γ_{top} and γ_{bottom}. A full derivation of Eq. (2) is given in the Supplementary Material. In this work, only the top half of the differential sensor is functionalised to allow for cancellation of the signal from the beads in suspension.

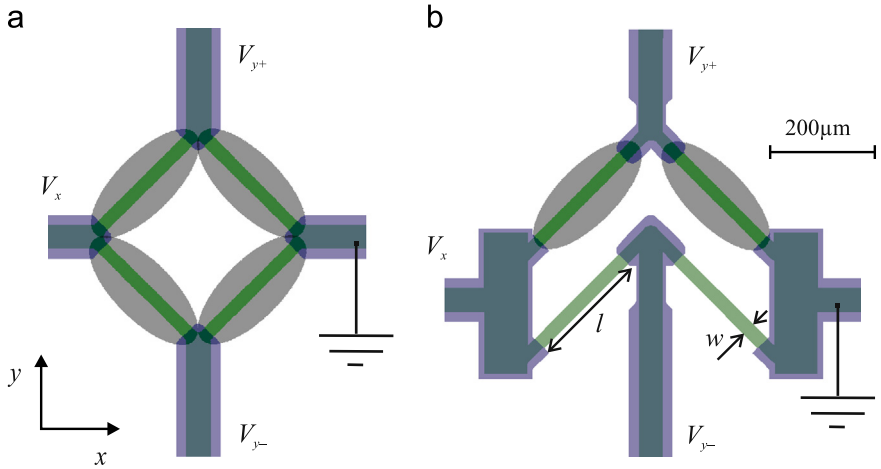

Fig. 1. Schematic illustration of sensor geometries: (a) PHEB sensor (b) Differential PHEB (dPHEB) sensor. The sensors are voltage-biased along the x-axis and the sensor signal V_y is measured along the y-axis. The arms of the sensor bridges are functionalised with surface-linked probes as indicated by the grey areas.