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On-chip magnetic bead-based DNA melting curve analysis using a magnetoresistive sensor

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ABSTRACT

We present real-time measurements of DNA melting curves in a chip-based system that detects the amount of surface-bound magnetic beads using magnetoresistive magnetic field sensors. The sensors detect the difference between the amount of beads bound to the top and bottom sensor branches of the differential sensor geometry. The sensor surfaces are functionalized with wild type (WT) and mutant type (MT) capture probes, differing by a single base insertion (a single nucleotide polymorphism, SNP). Complementary biotinylated targets in suspension couple streptavidin magnetic beads to the sensor surface. The beads are magnetized by the field arising from the bias current passed through the sensors. We demonstrate the first on-chip measurements of the melting of DNA hybrids upon a ramping of the temperature. This overcomes the limitation of using a single washing condition at constant temperature. Moreover, we demonstrate that a single sensor bridge can be used to genotype a SNP.

1. Introduction

Magnetic beads have the advantage over, e.g., fluorescent tags that virtually all biological and chemical samples do not exhibit significant magnetic properties and hence that there is no magnetic signal background from the sample matrix. In addition, the development of magnetoresistive sensor technology for read heads in hard disk drives has spawned extensive interest in the use of magnetoresistive sensors for magnetic biosensing [1–5].

DNA microarrays have revolutionized the analysis of genetic mutations related to disease diagnostics and a single microarray can be used to analyze up to 10,000 locations in the genome [6]. Microarrays rely on allele specific hybridization, where the fluorescently tagged target hybridizes to a set of surface-bound capture probes matching the wild type (WT) and mutant type (MT) variants of the gene of interest. The fluorescence from the set of microarray spots for a given gene can, after a washing step where weakly bound targets are washed off, be used to determine the types of the gene that are present in the sample (genotyping) by using a microarray laser scanner. To obtain a reliable genotyping, it is important to choose a combination of capture probe lengths and washing conditions that enable a clear distinction between matching and mismatching probe–target hybrids and for single nucleotide polymorphisms (SNPs), where the MT type target differs from the WT target by a single base, this can be challenging [7]. SNP genotyping is therefore often carried out in a homogeneous format, where the melting of DNA hybrids (giving a fluorescent signal) is investigated as function of temperature and the presence of a SNP can be observed as a temperature shift in the melting curve. However, such measurements only allow for a limited set of probes to be investigated.

Magnetoresistive sensors have the potential to be used for the investigation of the magnetic bead binding to a large set of capture probes [8] and may moreover provide a compact and relatively inexpensive set-up suited for use outside a specialized laboratory setting [9]. Sensors based on the giant magnetoresistance effect have previously been used to detect DNA by several groups – usually by detecting the signals due to magnetic beads bound to sensors functionalized with capture probes after incubation and washing [2,3,10,11]. Although on-chip magnetic field generators have been presented [12], the magnetic beads are usually magnetized using an external electromagnet [13–15]. In our previous work, we have demonstrated the detection of surface-bound magnetic beads to the so-called planar Hall effect bridge (PHEB) magnetoresistive sensors using only the magnetic field due to the sensor bias current required to excite the magnetic beads, thus eliminating the need for external electromagnets. Moreover, using a differential sensor geometry, we have recently demonstrated the detection of a SNP from the real-time sensor signals measured during and after a single washing step [16].

Here, we expand on our previous work by providing the first demonstration of on-chip measurements of DNA melting curves

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using a ramping of the temperature. To obtain reliable results, it is essential to properly correct the temperature dependence of the sensor and magnetic bead parameters and we therefore give a detailed presentation of the data treatment procedure. Finally, we show for the first time that a single sensor bridge functionalized with WT and MT probes on the two sensor halves can be used for the genotyping of a SNP.

2. Theory

The sensor geometry is shown in Fig. 1 and comprises four segments of the magnetoresistive stack arranged in a Wheatstone bridge geometry. When a low uniform magnetic field H_y is applied to the sensor in the y-direction, the resistance of each sensor arm is

$$R\left(\alpha = \pm\frac{\pi}{4}, H_y\right) = R_0 \mp S_0 H_y \quad (1)$$

where α is the angle that each bridge arm forms with the x-axis, R_0 is the resistance of the arm in zero magnetic field and S_0 is the low-field sensitivity [16]. When the bridge is biased by a voltage V_x, a variation of the resistances results in an output voltage V_y from the Wheatstone bridge. Moreover, when the sensor is biased with a voltage $V_x = \sqrt{2}V_{\mathrm{RMS}}\sin(2\pi ft)$, where V_{RMS} is the root mean square (RMS) amplitude of the voltage and f is the frequency, the current running in the sensor arms generates a small alternating magnetic field in the proximity of the sensor surface [17,18]. This self-field magnetizes magnetic beads in the vicinity of the sensor that give rise to an average magnetic field $H^{\mathrm{sf}} = \gamma I_{\mathrm{arm}}$ acting perpendicular to the arm, where I_{arm} is the current in the arm and γ depends on the amount and distribution of magnetic beads as well as the sensor geometry [16,17,19]. In addition to a contribution from magnetic beads, partial shunting of the sensor bias current in the antiferromagnet results in a sensor self-biasing, which is nominally eliminated in the differential PHEB (dPHEB) geometry of Fig. 1 [19]. The detection of magnetic beads using the sensor self-field eliminates the need for externally applied magnetic fields. The bead signal can be measured in the second harmonic out-of-phase lock-in signal, which for the dPHEB geometry can be written as [16]

$$V_2'' = -\frac{1}{8}S_0(T)\left(\frac{V_{\mathrm{RMS}}}{R_0(T)}\right)^2(\gamma_{\mathrm{top}} - \gamma_{\mathrm{bottom}}) + V_0(T) \quad (2)$$

where γ_{top} and γ_{bottom} depend on the amount of beads present over the top and bottom halves of the sensor bridge and V_0 is introduced to account for an offset in the second harmonic out-of-phase signal that would be zero for a perfectly balanced bridge. As discussed by Rizzi et al. [16], Eq. (2) shows that the bead contributions from top and bottom arms cancel out in a uniform bead background. When only the top half of the sensor is functionalized with capture probes, this allows to cancel out the signal from beads in uniform suspension over the sensor. Moreover, in the present work, the top and bottom halves of the sensor will be functionalized with two different capture probes to directly obtain the differential binding signal between the two probes.

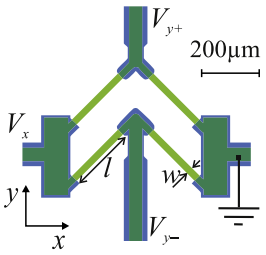

Fig. 1. Illustration of the dPHEB sensor geometry. The sensor is voltage biased along the x-axis and the voltage output V_y is measured along the y-axis. All sensors of the present study had $l = 250$ μm and $w = 25$ μm.

The signal V_2'' shows a non-trivial dependence on temperature. The low-field sensitivity $S_0(T)$ depends on temperature (T) and increases up to 10% when the temperature is ramped from room temperature to 70 °C and the increased temperature may also induce irreversible changes [20]. Moreover, the sensor offset $V_0(T)$ and the resistance $R_0(T)$ also vary with temperature. The terms γ_{top} and γ_{bottom} depend on the temperature stability of the binding of the magnetic beads as well as on the temperature dependent magnetic properties of the beads. Properly corrected as we will show below, however, the V_2'' data can be used to determine the stability of the binding of the magnetic beads with minimum influence from the other temperature dependent parameters.

3. Materials and methods

3.1. Sensor fabrication

The sensors of Fig. 1 with $l = 250$ μm and $w = 25$ μm were fabricated as described previously [16,21]. Briefly, the top-pinned magnetic stack Ta(5)/$Ni_{80}Fe_{20}$(30)/$Mn_{80}Ir_{20}$(10)/Ta(5) (thicknesses in nm) was sputter deposited. The easy axis of magnetization was defined during deposition by applying a saturating magnetic field along the x-axis. The electrical contacts of Ti(10)/Pt(100)/Au(100)/Ti (10) were deposited by electron beam evaporation. The sensors were passivated as described by Rizzi et al. [16] with a spin coated hybrid polymer (Ormocomp, Micro Resist Technology, GmbH, Germany) of thickness 900 nm. The wafer was diced into chips, each comprising six magnetic field sensors.

3.2. Surface functionalization

The allele specific DNA capture probes were covalently linked to the sensor surface through a silanization of the protective sensor coating as described by Rizzi et al. [16]. The DNA capture probes used in this work were designed by Petersen et al. [22] for SNP genotyping of the human beta globin (HBB) gene. The probes (sequences given in [16]) were purchased from DNA technology A/S, Denmark. Here, probes designed for the CD 8/9 mutation site were selected as a model system. The wild type (WT) and mutant type (MT) probes differ by a single base insertion. In addition, we used a biotinylated capture probe linked to the surface of a positive reference sensor to provide a direct linking of the streptavidin magnetic beads to the sensor surface. The capture probes were spotted over four dPHEB sensors as depicted in Fig. 2 using a Nanoplotter with a Nanotip (GeSim GmbH, Germany). The sensor surface was blocked prior to use in a solution of 1 mg/mL bovine serum albumin in 1 × phosphate buffered saline (PBS) for 20 min. The sensors will be named according to the probe used for functionalization (e.g., 'WT sensor' refers to the sensor functionalized with the WT capture probe).

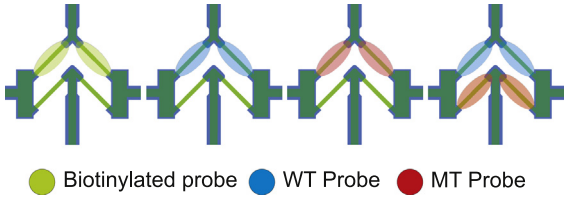

Fig. 2. Probe patterning for temperature denaturation studies. Sensors are functionalized with WT and MT DNA capture probes. A biotinylated capture probe provides a direct binding site for magnetic beads over the positive reference sensor.