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2012

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**Design Considerations for CMOS-Integrated Hall-effect Magnetic Bead
Detectors for Biosensor Applications**

by

Karl Ryszard Skucha

A dissertation submitted in partial satisfaction of the
requirements for the degree of
Doctor of Philosophy
in

Engineering – Electrical Engineering and Computer Sciences

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor Bernhard E. Boser, Chair

Professor Ming Wu

Professor Luke Lee

Fall 2012

**Design Considerations for CMOS-Integrated Hall-effect Magnetic Bead
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Karl Ryszard Skucha

Abstract

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Doctor of Philosophy in
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University of California, Berkeley

Professor Bernhard E. Boser, Chair

This dissertation presents a design methodology for on-chip magnetic bead label detectors based on Hall-effect sensors to be used for biosensor applications. Signal errors caused by the label-binding process and other factors that place constraints on the minimum detector area are quantified and adjusted to meet assay accuracy standards. The methodology is demonstrated by designing an 8,192 element Hall sensor array implemented in a commercial 0.18 μm CMOS process with single mask post-processing. The array can quantify a one percent surface coverage of 2.8 μm beads in thirty seconds with a coefficient of variation of 7.4%. This combination of accuracy and speed makes this technology a suitable detection platform for biological assays based on magnetic bead labels.

To my grandmother Stefania.

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Acknowledgements

I would like to first thank my family, namely my grandmother Stefania, my parents Bożena and Ryszard, and my sister Ewa and brother Tomasz, who have been instrumental in my development and education from the day I was born through the last days of my graduate studies. This work was made possible by their many sacrifices and continuous support of my efforts. I also want to thank my brother-in-law Bartek and sister-in-law Sylwia for their encouragement and my wonderful nieces Klaudia, Kaya, Zosia and my nephew Anthony for bringing joy to my life during my tenure at Cal.

My work was built upon the efforts and results of many others who have come before me, including Turgut Aytur, Mekhail Anwar, Prof. Eva Harris, Prof. Robert Beatty, and my advisor, Prof. Bernhard Boser, who had the vision to embark on this long-term research project. Special thanks also go to Octavian Florescu, who masterminded critical improvements and additional features that turned this project into a real product, further guiding and motivating my efforts.

My greatest thanks go to the ones whom I worked with on a day-to-day basis. Foremost, I thank Paul Liu, who was my partner in crime in designing the bead detector system and developing the relaxation detection method. I thank Mischa Megens for his expert advice on all things “physics” and for crafting the backbone of the MATLAB simulator often used in this work. I thank Simone Gambini for providing his expert chip design and circuits knowledge and for designing and testing the readout circuitry for the bead detector array presented in chapter 5. I thank Jungkyu Kim for providing his expertise in performing bioassays. I give thanks to Yida Duan, Igor Izyumin, Richard Przybyla, and Mitchell Kline for all their help, the interesting discussions we’ve had, and for all the fun times we had spent together. Finally, I thank my advisor, Prof. Bernhard Boser, for unrelentingly setting high standards on all my work and pushing my abilities to the limit.

I would like to express my gratitude to the BSAC staff, especially John Huggins, Richard Lossing, Alain Kesseru, and Helen Kim, who worked tirelessly to organize all the BSAC events and raise funding for BSAC researchers, myself among them. I thank the Microlab staff, including Bill Flounders, Sia Parsa, Joe Donnelly, Kim Chan, Marilyn Kushner, and the late Jimmy Chang, for their advice, training, and for providing me with the resources needed to complete my work. I want to also thank the EECS staff, including Patrick Hernan, Ruth Gjerde, and Shirley Salanio for providing the needed support in teaching and research matters.

Finally, I cannot express enough gratitude to my girlfriend, Haleh Razavi, without whom I would not be writing these words in the first place. She has incessantly encouraged me not to give up, even though I wanted to do so many times. Her presence in my life not only made this journey tolerable, but truly joyful, and I hope we can share that joy well beyond graduation day. Kheily mamnoon, Azize-Delam.

1 Introduction

Biochemical assays are used in medical diagnostic testing for many conditions including infectious diseases, heart attack, and cancer [1]. There is a growing need for technological solutions that enable diagnostic testing in diverse point-of-care (POC) settings, including local clinics in the developing world and physician offices and patients' homes in the developed world [2]. Specifications involving sensitivity, specificity, portability, affordability, and accuracy need to be met for each POC application without depending on existing laboratory infrastructure.

1.1 Immuno-Chromatographic strip tests

Currently the most common POC test is the Immuno-Chromatographic Test (ICT), which provides a very low cost and easy-to-use solution for qualitative testing. Its operation, presented in Figure 1-1, begins with the user providing a blood, saliva, or urine sample onto a sample pad at one end of a nitrocellulose strip. The sample may or may not contain a target analyte, which is the particular biological molecule, such as a protein, a nucleic acid, or a virus, that the test is configured to detect. After the sample is applied, capillary forces pull the sample through the conjugation pad where lyophilized labels, such as colloidal gold particles, fluorophores, magnetic particles, or other types of labels, re-solubilize and bind to target analyte in the sample. These labels are then pulled onto a test line coated with capture antibodies specific to the target analyte. Labels that bind to the target analyte will therefore bind to the capture antibodies immobilized on the test line, causing the normally invisible test line to become visible to the unaided human eye when sufficiently many labels bind. Unbound labels will continue down the strip and will bind to the control line, which captures labels regardless of whether the target analyte is present in the sample, serving as an indication of a valid test. A visible control line and a visible test line indicate a positive result, a visible control line and invisible test line indicates a negative result, and an invisible control line indicates an invalid test.

Despite their commercial success, ICTs suffer from low biochemical sensitivity, lack of inherent quantitation, and ambiguous results. The sensitivity of ICTs is limited to approximately 20-40 pM of analyte concentration in purified protein [3], [4] and to about 1 nM in raw samples [5]. This sensitivity is insufficient for many applications such as prostate cancer [6] and myocardial infarction (heart attack), where studies show that strip tests were inaccurate even 4 hours after the onset of symptoms [7], well after intervention needs to occur. Without embedded optical detectors or external readers that substantially increase test cost, strip tests can only be used for qualitative determination and are unsuitable for applications requiring quantitation. This leads to another problem: in cases where the test line is only partially visible, users are more likely to misinterpret the results [8]. While strip tests have been tremendously useful in many low-sensitivity POC

applications, they are unsuitable for a range of biomarker applications where more sensitive and/or quantitative detection are needed.

Figure 1-1: Immuno-chromatographic test operation.

1.2 Enzyme-linked immunosorbent assay (ELISA)

Many laboratory immunoassays used today based are based on colorimetric or fluorescent detection methods and provide sufficient sensitivity for the aforementioned applications [1], [3]. One example is the enzyme-linked immunosorbent assay (ELISA), which is shown in Figure 1-2. In a typical “sandwich” ELISA, a known concentration of capture antibody is bound to a surface, typically a polystyrene microplate or a gold-coated substrate. Subsequently, a sample potentially containing a target analyte is presented over the surface. Any target analyte present in the sample binds specifically to the surface via the capture antibody. Enzyme-linked secondary antibodies, also specific to the target analyte, are then added to the sample solution where they bind to the surface via another section (i.e., another epitope) of the already bound target analyte. Remaining unbound target analyte and enzymes are then washed away and the remaining density of enzymes bound to the surface is proportional to the original concentration of the target analyte in the sample. Next, a chemical substance is added that is converted, by the bound enzyme, into a colored or fluorescent substance. The surface concentration of the enzyme in the sample, and thus the concentration of the target analyte, can now be determined by measuring the change in absorbency or fluorescence of the sample.

ELISAs are generally regarded as the gold standard for immunoassays performed in laboratory settings [1], [2]. However, these assays are difficult to use in POC settings for two reasons. First, ELISAs require lengthy incubation times, particularly because it takes a long time for the target analyte to diffuse to the surface where it can bind to the capture antibody and also because enzymatic reactions typically take over an hour to produce detectable signals [1]. Second, optical detectors that are capable of detecting slight color changes are expensive to manufacture and thus are not compatible with disposable POC devices [2]. In particular, it is very difficult to measure color changes using complementary metal-oxide-semiconductor (CMOS) technologies without applying specific filters to filter out ambient light and, in the case of fluorescent detection, filters to block light used to excite fluorescent particles [2].

Figure 1-2: ELISA protocol.

1.3 Magnetic label immunoassay (MIA)

One approach to implementing assays in a small, inexpensive form factor that retains the high sensitivity protocol associated with ELISA involves using superparamagnetic microbeads as labels. Utilizing magnetic labels facilitates assay protocol integration as the labels can be detected electromagnetically rather than optically, obviating the need for optical components. This also enables integration with standard electronic processes since magnetic sensors and actuators used to detect and manipulate magnetic beads can be readily implemented in a CMOS process. A CMOS process provides a low-cost and robust solution for the detector component of a POC system and can be readily scaled to high volumes due to existing manufacturing capacity. Magnetic labels have several additional qualities that make them excellent candidates for POC applications:

- a) magnetic interference produced in biological systems and biological buffers is much smaller than the signal produced by the beads,

- b) magnetic beads are stable in biological solutions and in lyophilized form, enabling robust detection and long shelf life,
- c) magnetic beads are amenable to remote manipulation via electromagnetic means, which can enable integrated mixing and washing steps for a fast and highly integrated assay protocol [9], [10],
- d) single individual magnetic beads can be detected with high signal-to-noise ratio (SNR), enabling robust and rapid bead detection methods, and
- e) magnetic beads can be detected in opaque buffers.

Figure 1-3 shows an example of a sandwich immunoassay utilizing magnetic beads as labels that is performed on a microchip with integrated magnetic detectors. First, the surface of the chip is functionalized with capture antibodies that specifically bind to a target analyte being detected. In the second step, an aqueous sample containing the target analyte is introduced and the target analyte binds to the surface via the capture antibodies. Subsequently or concurrently, magnetic bead labels are introduced and bind to the target analyte via conjugated detection antibodies specific to another epitope of the target analyte. Any non-specifically bound beads may be removed using magnetic forces [9], [11] or hydrodynamic forces if the chip is integrated into a microfluidic system [12]. The remaining specifically bound beads can then be detected and used to infer the initial concentration of target analyte.

Figure 1-3: Magnetic immunoassay (MIA) protocol

On the surface the protocol associated with ELISA and MIA appears very similar. Aside from the obvious difference in type of label, one difference is the size of the label. Label size affects label diffusion behavior, binding behavior, and signal strength; changing the size of the label often requires changes in the entire assay protocol and is difficult to analyze beyond experimental means. Small labels used in ELISAs and other laboratory tests, such as fluorophores or enzymes, have dimensions that are similar to target analytes

such as antibodies, which are on the order of 5 nm. Labels much larger than the target analytes are much less typical. As will be explained in chapter 2, large labels require larger detector areas but have the advantage of being easily manipulated via electromagnetic or hydrodynamic forces to enable mixing and to increase surface contact, resulting in a faster assay protocol with integrated washing [10], [12]. Table 1-1 shows a comparison between typical specifications of commercial ELISAs and two published immunoassays utilizing labels on the order of a micrometer in diameter. The two large-label assays are on average twenty times faster than ELISA and have comparable detection limits, indicating that microlabels are especially suitable in situations where a fast assay protocol is important.

	ELISA	Assay 1 [10]	Assay 2 [12]
Label Size	5 nm – 15 nm	0.5 μm	2.8 μm
Detection Limit	100 fM – 10 pM	200 fM	1.7 pM
Assay Time	2 – 4 hours	4 minutes	13 minutes
Notes	Typical values	Magnetic mixing and washing	Hydrodynamic flow and washing

Table 1-1: Comparison of ELISA to assays utilizing microbeads as labels

One particular concern with using microbeads is that only tens to hundreds of magnetic labels may constitute a positive signal, whereas in ELISA the label count is in the millions for detectable concentrations. A result inferred from such a small number of labels may have significant errors caused by binding statistics and label variations. It is thus important to analyze and quantify these errors and design a detector system that achieves sufficient accuracy while keeping detection times and energy consumption levels within POC requirements.

This dissertation focuses on the analysis of error sources affecting the final detector signal and provides an example implementation of a compact and robust magnetic label detector based on CMOS-integrated Hall-effect sensors. In chapter 2, errors impacting the final signal are quantified and a constraint on the minimum required detection area is derived for a required level of signal accuracy. In chapter 3, magnetic labels and several state-of-the-art magnetic detectors are reviewed. Chapter 4 presents the architectural decisions for a Hall-effect sensor unit cell and provides results obtained from a test array. Chapter 5 extends the conclusions from chapter 4 to design and optimize a large array of sensors and readout circuitry; the chapter concludes with the associated measurement results and performance metrics.

2 System performance and error analysis

2.1 Overview

The primary goal of a point-of-care diagnostic assay is to provide an accurate reading of the concentration of the target analyte under certain constraints. While these constraints vary among different applications, in general, the detection component of a point-of-care tester needs to:

- a) be inexpensive as the total system cost is limited to a few dollars [13],
- b) be powered by a battery that is AAA or smaller to enable portability, and
- c) perform the detection step in less than one minute as the entire assay, including any necessary incubation steps, needs to complete in 5-10 minutes.

From these requirements we see that detection area, energy consumption, and readout time are relevant performance metrics that should be considered and minimized during the design process while maintaining a certain level of readout accuracy as determined by the application.

When an assay is performed many times under the same conditions (i.e., same analyte concentration, same temperature etc.), the results vary with a Gaussian distribution where the mean of the results is μ and the standard deviation of the results is σ . To quantify assay accuracy, commercial assay kits commonly provide the user with a standard curve describing the relationship between the output signal and the analyte concentration and a coefficient of variation (CV) defined as:

$$CV = \frac{\sigma}{\mu} \quad (1)$$

The CV determines the relative accuracy of an assay, while the standard curve allows one to map the output signal from the detector to the actual analyte concentration.

Assays for different applications have varying accuracy and dynamic range requirements. The dynamic range of an assay is defined as the ratio of the highest detectable concentration to the lowest detectable concentration. In some cases, as in a C-reactive protein (CRP) assays, high dynamic range is required as CRP levels vary by as much as five orders of magnitude [14]. In other cases, such as in hemoglobin A1c assays, high accuracy is required as differences of several percent result in different treatment plans [15]. In general, it is desirable to have a CV of 2% – 20% over several orders of magnitude of analyte concentration [16]. While both the standard curve and the CV are largely dependent on and often limited by the biological reagents used, it is important to ensure that the characteristics of the labels and the detection system do not unnecessarily degrade assay accuracy.

Before discussing error sources contributing to the CV, we need to relate the label signal, defined as the total signal from all labels situated on the sensors, to the analyte concentration being measured. In many assays this relationship is non-linear and, in the region between the limit of detection and saturation, can be described by a power law

given by:

$$[A] \propto n^x \quad (2)$$

where $[A]$ is the analyte concentration, n is the number of labels on the surface, and the exponent x is typically between 1-3 for published magnetic bead assays [12], [17], [18]. This non-linearity stems from the non-uniformity of binding affinities between individual analyte-antibody pairs and steric effects associated with the analyte and labels [19]. For small CVs, the non-linearity results in the relationship between CV_{label} and $CV_{[A]}$ of:

$$CV_{[A]} = x \cdot CV_{label} \quad (3)$$

The non-linearity in the standard curve effectively reduces the accuracy. For example, for a square law relation between labels and analyte concentration (i.e., $x = 2$), a CV_{label} of 10% results in a $CV_{[A]}$ of 20%.

2.2 Error sources

Several error sources are present in label detectors that can significantly impact the CV of the label signal. Some, like electronic noise, are present in all detector systems, whereas errors due to label variations, label binding location variations, and the label binding process are of particular concern in cases where a small number of labels constitute the final signal. Electronic noise comes from thermal noise and flicker noise generated by sensors and detection electronics. It is desirable to keep the flicker noise corner frequency below the measurement bandwidth and to average or integrate the signal to reduce thermal noise to the required level. Label variations arise from variations in label size and/or variations in the signal each label generates (e.g., variations in magnetic content for magnetic labels) and have a standard deviation between 10%–30% for magnetic beads used in this work. Label binding position variations come from location-dependent sensitivity of the detectors and depend on the sensor design, which is discussed in more detail in chapters 4 and 5.

In general, with a proper detector design, sufficient readout time, and suitable labels, the aforementioned error sources can be kept small. However, signal variations from the label binding process pose a fundamental error source in immunoassays. The binding of a capture antibody to its target analyte is a probabilistic event and follows dynamics that are common to all affinity-based biosensors and are known to generate biological shot noise [20]. Since each capture event is unlikely (esp. at low target analyte concentrations) and approximately independent of the others and since many such events occur, the number of labels bound to the sensor surface can be described by a Poisson distribution, where the standard deviation of the number of labels is equal to the square root of the mean number of labels. This error source is negligible in assays using large amounts of small labels such as enzymes or fluorophores, but in the case that only tens to hundreds of labels make up the final label signal, this error is significant and is further analyzed below.

Given that the number of labels bound to the surface for a particular analyte concen-

tration has mean n , then according to the Poisson distribution the standard deviation is $\sqrt{n}$ and the CV (CV_P) is $CV_P = \sqrt{n}/n = 1/\sqrt{n}$. Thus, the minimum number of labels required to achieve a certain CV_P is given by:

$$n = \frac{1}{CV_P^2} \quad (4)$$

For example, 400 labels are required to achieve a CV_P of 5%. This variation can be reduced only by increasing the number of bound labels, which, for a given analyte concentration, requires an increase of the sample volume and detection area.

Figure 2-1 (a) and (b) confirm the above analysis of Poisson error for magnetic bead label binding. A magnetic bead immunoassay was performed on a glass substrate using 100 ng/mL of mouse IgG as the target analyte. The total assay area was divided into multiple, equal-area regions (Figure 2-1 (a)) and the mean, standard deviation, and CV of the number of beads in the regions were calculated. The calculations were then repeated for smaller regions and the CV was plotted vs. mean number of beads per region in Figure 2-1 (b). A close correlation between the measured data and the theoretical Poisson distribution given by Eq. (4) is observed. This analysis effectively treats each region as a separate assay and rejects other variations, such as inconsistencies in sample preparation and delivery steps, variations in amounts of biological reagents used, and environmental effects, which would normally occur between different assays and further increase the CV. Thus the Poisson error sets the lower limit for the CV, and the final CV may be higher due to the aforementioned effects external to the detector system.

Figure 2-1: (a) An optical image of magnetic beads on a glass substrate where the assay area is divided into nine equal-area regions. (b) A graph showing the CV vs. the theoretical and measured mean number of beads per region.

Now that the error sources have been identified, a formal error analysis may be performed. Let σ_P , σ_{pos} , σ_{sig} , and σ_{noise} correspond to the standard deviation due to Poisson error, label binding position variations, label signal variations, and thermal electronic noise, respectively, normalized to the mean signal produced by one label. Assuming the

aforementioned error sources are independent and noting that $\sigma_P = 1$, the total CV (i.e., CV_{label}) is given by:

$$CV_{label} = \frac{\sigma_{label}}{n} = \frac{\sqrt{n + n\sigma_{pos}^2 + n\sigma_{sig}^2 + N\sigma_{noise}^2}}{n} \quad (5)$$

where n is the number of labels and N is the total number of sensors forming the detection area. Just like the Poisson error, the errors from label position variations and label signal variations scale with $\sqrt{n}$, whereas errors due to electronic noise depend on the total number of sensors and thus are constant for a particular number of sensors and a particular measurement time per sensor. Assuming electronic noise can be made small by averaging the sensor signals over a sufficiently long measurement time, the minimum number of labels required to attain a certain CV_{label} and $CV_{[A]}$ is given by:

$$n = \frac{1 + \sigma_{pos}^2 + \sigma_{sig}^2}{CV_{label}^2} = \frac{x^2(1 + \sigma_{pos}^2 + \sigma_{sig}^2)}{CV_{[A]}^2} \quad (6)$$

Recall that x is the exponent in the power law relationship between the number of labels and the analyte concentration, and as expected, Eq. (6) reduces to Eq. (4) when error sources from label position variations and label variations are negligible. For example, for $x = 2$ and assuming $\sigma_{pos} = 0.3$ and $\sigma_{sig} = 0.3$, which are the approximate values for the sensor array and labels presented in chapter 4, for a $CV_{[A]}$ of 10%, the minimum number of bound labels is $n = 435$.

2.3 Minimum detection area

The minimum number of labels and the required dynamic range of detection place a constraint on the minimum detection area. The dynamic range ($DR_{[A]}$), or the ratio of the highest quantifiable concentration to the lowest quantifiable concentration, needs to be between 100 and 10,000 for most immunoassays. Recall that because of the power law relationship in Eq. (2), the relationship between the dynamic range of the number of labels (DR_{label}) and $DR_{[A]}$ is:

$$DR_{[A]} = DR_{label}^x \quad (7)$$

For example, if $x = 2$, a DR_{label} of 100 results in a $DR_{[A]}$ of 10,000. The non-linearity of Eq. (2) effectively extends the dynamic range of the assay at the cost of decreasing the accuracy (i.e., by increasing the CV as shown in Eq. (3)).

The minimum detection area can be derived from Eq. (6) and Eq. (7). According to Eq. (6), at the lowest quantifiable concentration the assay needs at least n labels to keep

Poisson error within a required $CV_{[A]}$, whereas, according to Eq. (7), at the highest analyte concentration at least $n \times DR_{label}$ labels need to bind to the surface. Assuming that at saturation the surface is approximately fully covered with labels, the required detection area is:

$$A \approx d^2 \cdot DR_{label} \cdot n = \frac{d^2 \cdot DR_{[A]}^{1/x} x^2 (1 + \sigma_{pos}^2 + \sigma_{sig}^2)}{CV_{[A]}^2} \quad (8)$$

where d is the label diameter. Figure 2-2 shows the relationship between the minimum detection area and $CV_{[A]}$ for 2.8 μm diameter labels with $x = 2$, $DR_{[A]}$ of 10,000, $\sigma_{pos} = 0.3$, and $\sigma_{sig} = 0.3$.

Figure 2-2: Minimum detection area for an assay with a DR_{label} of 100 utilizing 2.8 μm labels vs. the desired CV.

Several interesting implications can be derived from Eq. (8) and Figure 2-2. First, the non-linearity in the standard curve actually helps to reduce the required detection area as the term $DR_{[A]}^{1/x} x^2$ decreases as x becomes larger for typical values of $DR_{[A]}$. Second, the detection areas for a high accuracy assays ($CV_{[A]} < 5\%$) utilizing 2.8 μm labels are on the order of 1 mm^2 and are readily attainable on CMOS chips. Labels larger than 10 μm , however, are not suitable for on-chip assays as they would require areas in excess of 10 mm^2 just for the sensors, a size that would be impractical due to high chip costs.

The above analysis derives the minimum required detection area as a function of label size and assay accuracy and is not limited to solely magnetic sensors and magnetic labels; it can be applied to any type of sensor or sensor array designed to detect labels, for example, fluorescent or colored beads in conjunction with optical detectors [21–23]. Since it is desirable to keep the detection area as small as possible, the errors from non-Poisson error sources should be made smaller or equal to the Poisson error during the design and detection processes. Additionally, the detection time and energy should also be minimized, which, for many sensor types, is most effectively achieved by maximizing the signal detected by each sensor. The next chapter will introduce the properties of magnetic bead labels and discuss state-of-the-art sensor technologies used for their detection. Chapter 4 will apply the analysis provided here and the properties of magnetic particles discussed in the next chapter to the design and optimization of Hall-effect sensors for the detection of magnetic bead labels.

2.4 Summary

Immunoassays are characterized by a standard curve that maps the output signal to the analyte concentration and a coefficient of variance (CV) that describes the normalized variations in the assay. A detector needs to be designed with a CV in a clinically relevant range (e.g., between 2% – 20%) over a clinically relevant dynamic range of analyte concentration (e.g., 100 – 10,000). Errors stemming from the detector, errors fundamental to the label binding process, and the finite size of the label set constraints on the minimum detection area, which is on the order of 1 mm^2 for high accuracy immunoassays utilizing micron-sized labels and can readily be implemented on CMOS chips.

3 Magnetic labels and magnetic detectors

Magnetic bead labels are abundant at biological laboratories and pharmaceutical companies as they are very useful in separating one molecule or cell from another in a solution, a process that is commonly performed. Due to their high prevalence, favorable properties presented in chapter 1, and enablement of highly integrated sensing systems utilizing magnetic sensors, they make excellent candidates for assay labels. In this chapter, we discuss the properties of magnetic assay labels and review various technologies used for their detection in bioassay applications.

3.1 Magnetic labels

Magnetic microbeads and nanoparticles have been widely used in biomedical applications, such as cell separation and medical imaging [24], and recently they have been utilized as labels in diagnostic tests [12], [17], [18], [25–27]. The magnetic beads used for this work are the popular and commercially available Dynabeads® (Invitrogen Inc, Oslo, Norway). These beads are made up of iron-oxide nano-particles enclosed in a spherical polymer matrix, as shown in Figure 3-1. The iron-oxide particles are on the order of 10 nm to 20 nm in diameter and the beads used in this thesis are between 1 μm to 4.5 μm in diameter [28]. In the absence of an external magnetic field, each magnetic nanoparticle forms a magnetic dipole with a magnetic moment pointing in a random direction. Consequently, the individual magnetic fields from each nanoparticle cancel out and the bead as a whole does not generate a measurable magnetic field.

Figure 3-1: Composition of a microbead and response to an external field.

Dynabeads display superparamagnetic properties, meaning they become magnetized in the presence of external magnetizing field. As shown in Figure 3-1, when an external field (B_{pol}) is presented over the bead, the dipole moments of the magnetic nanoparticles within the bead align themselves to the external field, generating an induced magnetic field (B_{bead}) that is superimposed with the external field. Stated another way, superparamagnetic beads amplify or concentrate external magnetic fields in the vicinity of the bead. The magnetization response is shown in Figure 3-2 for 2.8 μm and 4.5 μm Dynabeads. As the external field becomes larger, the induced field increases until saturation is reached, which results from the magnetic dipoles becoming fully aligned with the external field and occurs at approximately 100 mT. Magnetization curves for ferromagnetic particles generally show a hysteresis as remnant magnetic fields remain after magnetization. In contrast, the paramagnetic nanoparticles in Dynabeads display no detectable remnant field after the external field is removed since the magnetic dipoles relax back to a randomized state. This feature is very important for immunoassay applications since beads retaining a remnant field would attract each other, aggregating in clumps and skewing the post-assay signal.

Figure 3-2: Magnetization curve at room temperature for M-280 (left) and M-450 (right) [29].

Let us now consider the magnetization of the particles quantitatively. As discussed above, the particles' magnetic dipole moments m_p align more strongly with increasing magnitude of the external polarization field $\mathbf{B}_{pol}$ until the magnetization of the bead $\mathbf{M}_{bead}$ saturates. This behavior is described by a Langevin equation [30]:

$$\mathbf{M}_{bead}(\mathbf{B}_{pol}) = M_{sat} \left[\coth \frac{m_p B_{pol}}{kT} - \frac{kT}{m_p B_{pol}} \right] \frac{\mathbf{B}_{pol}}{B_{pol}} \quad (9)$$

where M_{sat} is the saturation magnetization of the bead and depends on the properties of the particles within the bead, k is the Boltzmann constant, T is the temperature, and μ_0 is the permeability of free space. For small applied magnetic fields, such as those generated on-chip, we can linearize the function and estimate using:

$$\mathbf{M}_{bead}(\mathbf{B}_{pol}) = \frac{\chi_{bead} \mathbf{B}_{pol}}{\mu_0} \quad (10)$$

where χ_{bead} is the susceptibility of the bead medium, which is 1.38, 0.76, and 1.63 for MyOne, M-280, and M-450 beads, respectively [29]. The magnetic moment of a bead is simply $\mathbf{m}_{bead} = \mathbf{M}_{bead} \mathbf{V}_{bead}$, and the induced magnetic field $\mathbf{B}_{bead}$ is given by the magnetic dipole equation:

$$\mathbf{B}_{bead} = \frac{\mu_0}{4\pi} \left[\frac{3(\mathbf{r} \cdot \mathbf{m}_{bead})\mathbf{r} - (\mathbf{r} \cdot \mathbf{r})\mathbf{m}_{bead}}{r^5} \right] \quad (11)$$

where r is the distance from the bead center to the point of measurement. This induced field is measured on-chip and used to detect the presence of a bead. For a B_{pol} on the or-

der of 1–10 mT, which are reasonable values for on-chip magnetic field generators, the induced B_{bead} is approximately 1–100 μ T at 3 μ m away from a micron-sized magnetic bead. For distances beyond the dimensions of the bead (e.g., 2–3 μ m for 2.8 μ m beads), the magnetic field magnitude decays with $1/r^3$.

Several implications can be obtained from the above equations. First and most obvious is that larger beads have a larger magnetic moment and generate a larger B_{bead} . However, the size of a bead is limited by the fact that the centers of larger beads are further away from the detectors and the effect of decaying bead field with distance eventually cancels out the increases in bead volume. On the other hand, as was discussed in chapter 2, larger beads require a larger detection area, which is unfavorable for low-cost designs. The second implication is that a larger B_{pol} produces a larger B_{bead} , though diminishing returns set in at about $B_{pol} = 10$ mT and the increase in B_{bead} becomes negligible at $B_{pol} > 100$ mT. Increasing B_{pol} generally requires more power consumption in the external magnetic field generator or the magnetic field generator must be brought closer to the bead; the latter approach is used in this thesis and is further explained in chapter 4. Third, it is important for sensors to be placed as close to the beads as possible since the bead field decays rapidly with increasing distance. This is achieved via a post-processing step described further in chapter 4.

3.2 Magnetic label detectors

Now that the properties of magnetic beads and their induced magnetic fields have been explained, the next step is choosing an appropriate technology for their detection. The detector needs to convert the magnetic signal from the bead, B_{bead} , into an electrical signal that indicates the presence or absence of the bead. Since our goal is a POC detector system, we will only consider technologies that can be integrated into microchips.

The three main technologies for integrated magnetic bead detection are GMR sensors [17], [25], [26], inductors [27], and Hall-effect sensors [11], [18], [31–35]. GMR sensors are based on the recently discovered giant magnetoresistance effect and offer excellent sensitivity. However, GMRs saturate at very low fields and require uniform polarization fields to prevent saturation, which can only be reliably produced externally. Even highly integrated external electromagnets used in conjunction with GMRs dissipate on the order of 1 A of current [36]. Furthermore, GMRs are made using ferromagnetic materials not standard in CMOS process and thus generally must be made on a separate chip, which limits functionality and reduces integration. On-chip inductive sensors also offer excellent sensitivity by utilizing frequency-shifting techniques and quality-factor amplification. However, because on-chip inductors need to be tens of microns in diameter to achieve good quality factors, their signal-to-baseline ratio is on the order of several ppm and thus extensive calibration steps are required [27]. Further, since inductors operating at 1 GHz are sensitive to conductive media, there exists potential interference from non-magnetic sources. Hall-effect sensors, which are utilized in this thesis, have relatively poor sensitivity but are robust; they are not sensitive to conductors and they can be used in conjunction with integrated electromagnets generating non-uniform magnetic fields.

Further, these sensors can be scaled to several micrometers in size and arrayed to arbitrarily large detection areas, which enables detection of a single bead in a very large area. This also allows the sensors to achieve signal-to-baseline ratio on the order of 1%–10% for magnetic microbeads using polarization detection [13] and over 100% using relaxation detection techniques [33], [34]. These detection methods are explained below.

3.3 Detection methods

Detecting the magnetic field from a bead in the presence of a much larger magnetizing field requires careful choice of a detection algorithm and associated circuitry. For example, a typical $2.8\ \mu\text{m}$ diameter bead with susceptibility $\chi = 1$ in a 3 mT external field generates less than $15\ \mu\text{T}$ of induced field $4\ \mu\text{m}$ away from the bead center. This induced magnetic field from the bead, B_{bead} , is nearly 50 dB lower than the magnetizing field B_{pol} . This constraint imposes stringent requirements on a detector's dynamic range, offset, linearity, and stability with respect to environmental changes.

One approach to detecting B_{bead} is to set a threshold above B_{pol} and below $B_{\text{pol}} + B_{\text{bead}}$ and determine the presence of a bead based on whether a signal is above or below this threshold (Figure 3-3). This is referred to as the polarization detection method. To reject B_{pol} , reference sensors and/or pre-measurement calibration can be used [13], [31], resulting in a 40-60 dB reduction in B_{pol} . In a laboratory environment, using polarization detection conjunction with Hall sensors leads to successful detection of $2.8\ \mu\text{m}$ and $4.5\ \mu\text{m}$ beads [31]. However for smaller beads, like the $1\ \mu\text{m}$ MyOne beads, this technique is not reliable. In addition, variations due to environmental changes and user handling in POC settings are expected to be larger than in the laboratory, so it is likely that more advanced matching techniques and active temperature stabilization may be required, which add substantial overhead to the system.

Figure 3-3: Conventional label detection method based on magnetization/polarization.

Another approach to bead detection is detecting a bead's signature (i.e., some property a bead possesses that external magnetic fields do not). In this work we utilize magnetic relaxometry to detect a bead (Figure 3-4) [32], [34], [35]. As in polarization detection, the bead is first magnetized by an external field, B_{pol} . Then the polarizing field is switched off rapidly (e.g., with a time constant on the order of a few nanoseconds). The bead retains a remnant field for some time, which is sampled as soon as the polarizing field decreases to lower negligible levels, which effectively eliminates the baseline error due to B_{pol} .

Figure 3-4: Relaxation detection method.

The time constant of the remnant field of a monodisperse bead made up of magnetic nanoparticles of volume V is given by the Néel relaxation equation:

$$\tau_N = \tau_o e^{KV/k_B T} \quad (12)$$

where τ_N is the Néel relaxation time constant, τ_o is a constant on the order of 1 ns, K is a constant that depends on the properties of the bead, k_B is the Boltzmann constant, and T is the temperature. The constant K depends on many factors including material type and density of the particles and generally needs to be determined via measurement. An ideal monodisperse bead will be characterized by an exponential decay with a certain time constant and should be modulated at a frequency close to that time constant to achieve an optimal signal-to-noise ratio ratio divided by measurement time (SNR/T_m). The Dynabeads used in this work are composed of nanoparticles of different sizes approximately following the log-normal law [32]. The response of those beads can be thought of as a linear combination of Eq. (12) across the distribution of nanoparticle volumes within the bead. Consequently no single time constant exists and the bead displays an effective

time constant that is very highly dependent on the amount of time a bead is polarized. Thus the optimal frequency of modulation in terms of SNR/T_m is very shallow for Dynabeads and several other bead types that were measured across frequencies from 1 kHz to 10 MHz. In practice, the frequency of modulation is chosen to be higher than the $1/f$ corner frequency to eliminate $1/f$ noise stemming from electronics and external disturbances, and is on the order of 1 MHz for the system presented in chapter 5.

On-chip relaxation detection leverages the short time constants and miniaturized components achievable in modern sub-micron CMOS technology. A 50 dB reduction in baseline signal has been achieved this way without requiring baseline calibration or reference sensors [33]. Still, it needs to be noted that this technique is orthogonal to the calibration/reference sensor techniques presented earlier; both calibration and relaxation can be used in conjunction for even better baseline rejection [34]

3.4 Summary

Magnetic labels are ideal candidates for a POC label detector due their high prevalence, compatibility with integrated magnetic sensors, and high stability and signal fidelity in biological environments. The beads need to be paramagnetic to prevent clumping and thus require an external polarization field in order to be detected.

Out of several magnetic sensor technologies, we chose Hall-effect sensors due to their compatibility with CMOS, scalability to micron-sized levels, and good robustness in terms of specificity to magnetic fields and signal-to-baseline ratio. In order to further reject the external polarization field, a relaxation detection method was developed and utilized that detects a bead's remnant relaxation signal after the polarization field is turned off, reducing the baseline signal 300 fold.

4 Hall-effect bead detector design

As discussed in the previous chapters, Hall-effect sensors are can be integrated with CMOS-technology to enable compact and low cost bead detection systems. This chapter presents the concepts behind Hall effect and high-level design decisions made in developing the bead detector architecture and measurement algorithm. The chapter concludes with an implementation and characterization of an 8×8 sensor test array that is used to make more informed design decisions for a larger array presented in chapter 5.

4.1 Hall effect

The underlying principle behind Hall effect is the Lorentz force, which states that moving electric charges experience a force in the presence of a magnetic field that is orthogonal to the motion of charges and the direction of the magnetic field [38]. When charge flow is restricted to plates of conductive materials, this force manifests itself as an electric field across the plate that can be measured using electronic means.

Consider a flat, rectangular conductive plate having mobility μ_H that is biased by a voltage bias V_{bias} on two ends, as shown in Figure 4-1. Under steady state conditions, positive charges flow from the positive terminal to the negative terminal uniformly over the plate and no potential difference exists over sense contacts situated in the middle of the plate. Now consider applying a magnetic field B orthogonal to the plate. By the right hand rule, the magnetic field exerts a Lorentz force on the positive charges that points in the direction of the upper sense contact (relative to the Figure 4-1). As the positive charges are pushed towards the upper sense contact, they aggregate since they cannot exit the boundary of the plate. The converse occurs with negative charges; they aggregate on the lower sense contact. Thus a charge difference forms between the sense contacts and generates a potential difference V_H . The magnitude of V_H is directly proportional to the applied field B and is given by:

$$V_H = \frac{W}{L} G \mu_H V_{bias} B \quad (13)$$

where W and L are the width and length of the Hall plate, respectively, and G is a geometric factor having a value between 0 and 1.

Figure 4-1: The principle of Hall Effect.

The sensitivity of a Hall sensor is strongly dependent on the mobility of the material. In a standard CMOS process, Hall sensors are best realized with high mobility semiconductive structures such as n-wells, p-wells, transistors, or diffusion resistors, as opposed to low mobility metal plates. The sensitivity depends the term $W/L \times G$. This term is maximized when the Hall-effect sensor is approximately a square. Specifically, W/L should be set between 0.66 and 1.2, for which $W/L \times G$ is maximum and approximately equal to 0.6 [37].

4.2 Bead detector architecture

As discussed earlier, since magnetic beads used in assays are paramagnetic, a bead detector will need both a detector and a source of magnetic field to polarize the beads. A general structure of a Hall sensor bead detector is shown in Figure 4-2. A Hall sensor having a length L is located at a distance Z_2 below the chip surface. In order to obviate large, high-power external electromagnets, the polarization field generator is integrated on-chip. Wires located at distance of Z_1 below the surface carry currents $(+I_{pol}, -I_{pol})$ to generate a magnetic field (B_{pol}) that magnetizes the magnetic beads bound to the surface. The magnetized bead creates an induced magnetic field, B_{bead} , that is well modeled by a simple dipole [11] located at a height of $Z_2 + d/2$ above the sensor, where d is the bead diameter. The Hall sensor converts the vertical component of B_{bead} into a voltage signal of magnitude V_H , which appears across two sense contacts placed on opposite sides of the Hall sensor. In the CMOS process used in this work, $Z_1 = 1.1 \mu\text{m}$ and $Z_2 = 3 \mu\text{m}$, whereas L is left as a design parameter and is discussed further in this chapter and in chapter 5.

Figure 4-2: Cross-section of a Hall-effect bead detector showing relevant magnetic fields.

As discussed in chapter 2, detection areas on the order of 1 mm^2 are required when using microbeads as labels. Unfortunately, millimeter-sized Hall sensors are not sufficiently sensitive to detect magnetic beads without resorting to impractically lengthy signal averaging times and complex calibration steps. Although there is considerable local magnetic field arising from a magnetic dipole moment of a single bead, the total magnetic field integrated over the plane of the Hall sensor approaches zero as the plane becomes larger since magnetic field lines always form a closed curve [2]. Only a small Hall sensor can “capture” the local magnetic field lines from a dipole before they reverse direction. This concept is further illustrated in Figure 4-3(a). Consider a circular surface of radius r positioned $5 \text{ }\mu\text{m}$ below the center of a magnetized bead, which is a typical height from a bead center to the surface of a Hall sensor when the system is implemented in a CMOS process. As r is increased, the average magnetic field (Figure 4-3(c)) decreases because less flux per surface area passes through the circular surface (Figure 4-3(b)). To first order, the response of a Hall sensor is proportional to the average magnetic field orthogonal to the sensor [37], so Figure 4-3(c) also serves as an estimate of the output signal vs. the size of the Hall sensor. Large Hall sensors capture much less average magnetic field than small Hall sensors positioned directly over the bead. Optimally the Hall sensor needs to be infinitesimally small to capture the largest average field. Since this is unachievable in any CMOS process, Figure 4-3(c) highlights the range of loop radii that produce an aver-

age magnetic field within 20% of the maximum, which is approximately from 0-2 μm . Consequently, Hall sensors with dimensions up to 4 μm on each side produce relatively little signal loss while maintaining low sensor offset and good signal uniformity over the unit cell, as we will see further discuss in section 4.6.

Figure 4-3: (a) Schematic showing magnetic field lines from a magnetized bead passing through a loop of radius r , (b) total magnetic flux passing through the loop vs. radius r of the loop, and (c) average magnetic field passing through the loop vs. radius r .

From the analysis presented above and in chapter 2, it is clear that large arrays of micrometer-sized Hall sensors are needed to achieve a sufficient detection area for bead quantification. Another benefit of an array implementation is that polarization field generators can be placed in close proximity to magnetic beads, generating significant magnetic fields in the range of millitesla with currents on the order of milliamps.

4.3 Post processing

To first order, the magnitude of B_{bead} is inversely proportional to the cube of the distance between the bead center and the sensor. This relationship is shown in more detail in Figure 4-4 and shows that significant increases in signal strength can be obtained by reducing the distance between the bead and the sensor. Thus one of the primary design optimizations is to make this distance as small as possible.

In the sensor arrays presented here, the distance between the chip surface where the beads reside and the embedded sensor was reduced from 9 μm to 3 μm by etching of the inter-layer dielectric (ILD) above the sensor area, which results in nearly a 10x signal strength increase. Further etching is limited by the ILD thickness around metal 1 since this metal is needed for signal routing.

Figure 4-4: Magnetic field strength of a bead placed directly over a Hall sensor vs. the distance of the bead surface from the Hall sensor.

Figure 4-5 shows the post-processing flow [35]. Metallization layers, fabricated as part of the CMOS process, were used as a hard mask for the reactive-ion etching of the ILD. Individual chips were mounted onto a 6" wafer using photoresist and the pads of the chip were covered with photoresist that was applied manually using a thin brush. The photoresist was hard baked for 1 hour at 120°C. The wafer was placed in an RIE chamber (Centura Platform System MxP+) to remove the exposed ILD, which is a combination of silicon oxide and silicon nitride. The chamber was filled to a pressure of 200 mTorr and 150 sccm of Ar, 15 sccm of CF₄, and 45 sccm of CHF₃ were introduced into the chamber. The plasma was fired to 700 W for 30 seconds and the chamber was allowed to cool for 30 seconds afterwards. The firing of plasma and subsequent cooling was repeated approximately 40 times until all the ILD was etched off and the underlying metal 2 hard mask was exposed, which covers the sensor region. The wafer was inspected periodically and the process was deemed complete when no residual ILD over metal 2 was visible.

A metal wet etch was performed after the ILD etch by dipping the resulting wafer in an aluminum etch solution (80% H₃PO₄, 5% HNO₃, 5% CH₃COOH, and 10% DI, Transene Company Inc.) for 5 minutes to remove the metal hard mask, which brings the chip surface another 0.5 μm closer to the Hall sensors. Subsequently, the wafers were rinsed and a 2 minute dip in standard clean solution (5:1:1 ratio of H₂O, H₂O₂, and NH₃OH) was performed, which removes the Ti/TiN liners and makes the sensors optically visible. The wafer was again rinsed and the photoresist was stripped using PRS-3000 (J.T. Baker) heated to 80°C. The individual ICs were carefully removed, rinsed in DI water, and wire bonded to a package for testing.

After post-processing, only one metal layer and one poly-silicon layer remain for routing signals in the detection area, restricting the architecture of the polarization field generator and the sensor cell to only these two routing layers. It is important to note that although in this work the etching steps were performed after the CMOS process was completed, the etching steps can be readily integrated into the CMOS process by simply lengthening the time of the final passivation etch used to expose the pads.

Figure 4-5: Etching steps performed after CMOS IC fabrication to reduce the distance between the beads and the Hall sensors; a) chip is fabricated with a metal hard mask defining the sensor region; b) RIE etch is used to remove ILD over the sensor region; and c) exposed metal layer over the sensor surface is removed with a wet etch.

4.4 Polarization field generator architecture

The magnetic moment induced on a bead is proportional to the external field B_{pol} . As a result, placing polarization field generators in close proximity of the bead and increasing I_{pol} results in a larger magnetic moment and hence larger signal. The ability to place polarization field generators within several micrometers of the label is a critical advantage of fully integrated detectors over ones that resort to external magnets which typically consume on the order of 1 W of power [36].

Figure 4-6: Polarization field generators based on: a) polarization wires and b) polarization coils.

Two potential designs for magnetic field generators were analyzed; they are based on integrated polarization wires and integrated polarization micro-coils (Figure 4-6). In both architectures, an entire row of sensors is polarized at the same time to enable parallelized sensor readout. Polarization coils were implemented in [31] and their magnetic field is described by:

$$B_{pol,r} = \frac{\mu_o I_{pol} z}{2\pi r \sqrt{(a+r)^2 + z^2}} \left[E(k) \frac{a^2 + r^2 + z^2}{(a-r)^2 + z^2} - K(k) \right] \quad (14)$$

$$B_{pol,z} = \frac{\mu_o I_{pol}}{2\pi \sqrt{(a+r)^2 + z^2}} \left[E(k) \frac{a^2 - r^2 - z^2}{(a-r)^2 + z^2} + K(k) \right] \quad (15)$$

where μ_o is the permeability of free space, I_{pol} is the polarization current, a is the radius of the coil, r is the distance from the center of the coil to the axis of observation, z is the vertical offset from the plane of the coil, $E(k)$ and $K(k)$ are the complete elliptical integral functions of the first and second kind, respectively, and k is given by:

$$k = \sqrt{\frac{4ra}{(a+r)^2 + z^2}} \quad (16)$$

For polarization wires, the magnetic field is described by:

$$B_{pol,y} = \frac{\mu_o I_{pol}}{2\pi} \left[\frac{z}{z^2 + (a-y)^2} - \frac{z}{z^2 + (a+y)^2} \right] \quad (17)$$

$$B_{pol,z} = \frac{\mu_o I_{pol}}{2\pi} \left[\frac{a-y}{z^2 + (a-y)^2} + \frac{a+y}{z^2 + (a+y)^2} \right] \quad (18)$$

where μ_o is the permeability of free space, I_{pol} is the polarization current, a is half the spacing between the magnetization wires, y is the horizontal offset from the line directly in between the wires, and z is the vertical offset from the plane of the wires.

To quantify the performance of each architecture, the Hall voltage signal resulting from a magnetic bead that is magnetized with each polarization field generator was calculated. In order to make a fair comparison, both the radius of the polarization coil and the spacing between the two polarization wires were set to length a . The sensor pitch was also set to $3a$ in both cases so that each coil is a away from the next coil to allow for sufficient space between the coils. The polarization field, B_{pol} , was calculated at the bead center based on Eqs. (8)-(13). The magnetic bead was modeled as a dipole located in the center of the bead and the total z -component of the bead magnetic field, B_{bead} , was calculated at the Hall sensor. Since the bead may land off-center on a unit cell, the results presented below are averaged over the entire unit cell.

Figure 4-7 shows the relative difference in the average signal from a bead over the sensor unit cell for the two polarization field generators as a function of z/a , where z is the distance between the bead center and the coil/wire. The relative signal ratio is independent of the actual values of z and a (i.e., it just depends on the ratio z/a) and is within 10% of that shown in Figure 4-7 for values of d (i.e., the distance between the Hall sensor and the polarization coil/wire) from $0.5 \mu\text{m}$ to $4 \mu\text{m}$, which is a typical distance between the first metal layer and the silicon surface for most CMOS processes. As expected, polarization coils have a larger signal for beads in close proximity to the coil. This is because, unlike the wires, the coil surrounds the bead from all directions and thus generates a larger B_{pol} in the plane of the coil. On the other hand, polarization wires yield a higher signal when the bead dipole is over $0.8 z/a$ away from the coil. This may be understood by noting that from Eq. (14)-(18), to first order, the field due to a coil at distance z above the plane of the coil is proportional to z^{-3} , whereas the field due to polarization wires is proportional to z^{-2} , and thus decreases less with vertical distance. It is important to note that the results presented here are not only valid for Hall sensors but also for any type of magnetic sensor that is sensitive to vertical magnetic fields and fits within the boundary of the polarization field generator (i.e., its dimensions are smaller than $2a$).

Figure 4-7: Ratio of the average Hall voltage signal of the polarization wire architecture to the polarization coil architecture as a function of the ratio of z/a , where z is the vertical distance from the bead center to the plane of the polarization coils/wires and a is the radius of the coils or half the distance between polarization wires.

Figure 4-7 also shows points where different sized beads fit on the curve specific to our CMOS architecture. Although the surface of the chip is $1.1 \mu\text{m}$ away from the wires/coils, due to differing bead diameters, the bead centers are at different vertical distances from the wires/coils. On average, polarization wires generate larger signals for $2.8 \mu\text{m}$ and $4.5 \mu\text{m}$ beads, whereas for $1 \mu\text{m}$ beads and smaller, polarization coils generate larger signals.

In addition to the analysis presented above, there are few other considerations for deciding which polarization field generator to choose. First, the analysis above assumes that the polarization coils are ideal, whereas real coils are not complete circles and are not perfectly circular, leading to approximately a 5-10% smaller signal than calculated in Figure 4-7 (which depends the wire thickness and the process design rules). Second, because there are regions in between the coils where the bead is barely magnetized, the signal from polarization coils has approximately a 50% larger standard deviation with respect to bead position than the signal from polarization wires. This will effectively increase σ_{pos} in Eq. (8) and consequently a larger chip area will be required to maintain the

same detector system accuracy. Third, polarization coils have approximately a 30% greater resistance per unit cell and thus they can polarize only about 70% of the sensors that can be polarized with polarization wires at a given supply voltage. Thus detection will be approximately 30% slower using coils rather than wires. Fourth, polarization wires can be shared among different rows (i.e., the top wire of one row may be used as a bottom wire of the row above it), whereas this is not feasible with polarization coils. Separate coils would need to be implemented for each row, taking up approximately 10–30% more area. Lastly, it is easier to route other signals (e.g., supply and ground lines, word lines, etc.) next to the straight wires in the polarization wire architecture than the curving coils in the polarization coil architecture, leading to a less complex layout.

Given the results Figure 4-7 and the arguments presented above, polarization wires are preferable for detecting large beads and in general are more practical to implement in the CMOS process. Consequently, the polarization wire magnetic field generator was selected for the arrays presented in this thesis.

4.5 Sensor non-idealities and measurement algorithm

As discussed earlier, the magnetic bead signal B_{bead} is relatively small (around 10–100 μT) and can be corrupted by various sources of errors such as variations from sensors, circuitry, and the environment. In this section, we outline these error sources and present a measurement algorithm that significantly attenuates them.

First, miniaturized Hall sensors have an intrinsic offset that is on the order of 1% of V_{bias} (e.g., approximately 20 mV for a 2 V bias voltage) for the CMOS process used in this work. The equivalent input referred offset can be as large as 300 mT, over 10,000 times higher than the smallest bead signal we want to detect. Further contributing to the low frequency offset are interference from DC magnetic fields such as the Earth’s field ($\sim 50 \mu\text{T}$) and AC magnetic fields such as those from power lines and urban noise, but these error sources are significantly smaller.

Second, in the case polarization currents are switched on and off (which is the case for relaxation detection shown in Figure 3-4), substantial heating can occur in the current lines that generates temperature gradients over the sensors. Figure 4-8 (a) and (b) show simulation of temperature rise due to a polarization current of 32 mA flowing through the polarization wires. Specifically, Figure 4-8(a) shows a cross section displaying steady state temperature changes when the polarization line is turned on and Figure 4-8(b) shows a top view of an entire row of sensors displaying the temperature changes of the silicon substrate (where the Hall sensors lie). The highest gradients occur at the edges of the row where the polarization wires terminate and the heating effect stops (in reality the wires are routed to a much wider and higher level metal, which generates substantially less heat on the silicon). These temperature gradients manifest themselves as voltages on the Hall sensors via thermo-electric effects. Specifically, in silicon, the thermopower coefficient is 0.5 mV/K or about 8 mT/K when input referred to the magnetic field over Hall sensors. Thus a gradient of 1/1000 of a Celsius over a n-well Hall sensor can create

an input referred thermal gradient of $8\text{ }\mu\text{T}$, a disturbance that is similar to the smallest expected B_{bead} .

Figure 4-8: Thermal simulations of the bead detector: (a) cross section across a single row of sensors showing temperature gradients from the polarization lines to the silicon substrate, (b) top view of a $200\text{ }\mu\text{m}$ current line showing thermal gradients in the silicon substrate below the line.

The fourth source of error to be considered for measurement is the thermal and flicker noise from the sensor and electronics. Thermal noise sets the minimum integration/averaging time for obtaining a certain signal fidelity or signal-to-noise ratio (SNR), whereas flicker noise dictates the minimum required modulation frequency to make it negligible as compared to the thermal noise.

Now that we've discussed relevant error sources, we proceed to describing methods to attenuate them. In order to reject the sensor offset, 1/f noise, stray magnetic fields, and thermal effects, we utilize a correlated double sampling (CDS) signal measurement scheme shown in Figure 4-9. In phase 1, the bead is polarized over time t_m with external polarization field B_{pol} . In phase 2, B_{pol} is abruptly turned off but the polarization field from the bead, B_{bead} , remains. The Hall sensor signal is integrated over time t_1 , which starts 50-100 ns after B_{pol} is turned off to ensure there is no coupling of B_{pol} to the signal. In phases 3 and 4, the same process is repeated as in phases 1 and 2 except the B_{pol} is reversed and the Hall sensor signal, which is now the opposite of the signal at time t_1 , is integrated over time t_2 . The negative sample at t_2 is subsequently subtracted from the sample at t_1 , effectively adding constructively, to achieve a final signal. This procedure effectively eliminates sensor offset, 1/f noise at lower frequencies than the frequency of excitation, low frequency stray magnetic fields, and thermal effects. The effective signal before CDS and after CDS is shown in Figure 4-10, where in the post-CDS signal, relaxation behavior is clearly distinguishable. To attenuate thermal noise, the process in Figure 4-9 is repeated over multiple cycles and the samples are averaged, reducing thermal noise amplitude by a factor of 2 every time measurement time increases by a factor of 4.

Figure 4-9: Measurement algorithm for detecting magnetic beads.

Figure 4-10: Example of a signal before CDS (blue) including both offset and thermal effects and after CDS (red), where DC offset and thermal effects are cancelled.

4.6 Test array implementation and results

In order to test the Hall sensor bead detector and compare two Hall sensor plate types, one using an n-well and the other using a MOS active region, 8×8 sensor arrays were designed and implemented in the $0.18 \mu\text{m}$ CMOS process. Figure 4-11 shows the unit cell architecture for each implementation. Each unit cell contains a Hall plate, bias wires, polarization wires, signal bit lines, and, in the n-well implementation, access transistors similar to those in SRAM.

Figure 4-11: Unit-cells of both the n-well and MOS Hall sensor array implementations.

For the comparison, the size of each Hall sensor was chosen to be $4 \times 4 \mu\text{m}^2$. As shown earlier in Figure 4-3, larger Hall sensors generate a substantially smaller signal whereas smaller Hall sensors are difficult to implement in the CMOS process used in this work and generate only modest signal gains at the cost of higher sensor offset and larger signal variations. The Hall sensors are biased at the 2 V supply to maximize their sensitivity. The differential Hall voltage signal is routed perpendicular to the other wires using silicided poly-silicon bit lines. A digital word line connects to the gates of these access transistors or directly to the gates of the MOS Hall sensors to turn on an entire row of sensors for readout. In the MOS implementation, sense contacts of the Hall-effect sensor are placed slightly closer to the drain contact in order to increase the sensitivity [38]. Polarization current was chosen to be 32 mA for this comparison and the sensors/beads were magnetized with a frequency of 62.5 kHz.

Table 4-1 lists several parameters and measurement results obtained for each Hall sensor implementation. The highest Hall voltage signal and SNR are achieved when a bead lands directly over a sensor, while the lowest SNR occurs when a bead lands directly in between two pixels on the H-H' line. N-well sensors have over two times better sensitivity than MOS sensors but consume three times more power. Nevertheless, n-well Hall sensors achieve a better energy and time performance per detection event than the MOS sensors for the same level of minimum SNR (i.e., same σ_{noise}). At the minimum SNR of 14 dB, the probability of missed detection of a bead (false negative) is only 0.6%.

Parameter	Hall sensor	
	n-well	MOS
Polarization Current I_{pol} (mA)	32	32
Frequency f (kHz)	62.5	62.5
Sensitivity (V/V/T)	0.029	0.014
Sensor Bias Current (mA)	2.1	0.7
Measurement Time (ms)	70	350
Energy per detection (μ J)	290	490
Max. Hall Voltage V_H (μ V)	0.8	0.35
Max. SNR (dB)	23	17
Min. SNR (dB)	14	14
Probability of detection error	0.6%	0.6%

Table 4-1: Summary of sensor parameters for n-well and MOS Hall sensor.

To further evaluate the impact of non-uniform sensitivity across the unit cell, label binding position variations, σ_{pos} , were calculated for both sensors. The signal from one magnetic bead as a function of distance from the sensor center was measured and simulated using a FEM simulator implemented in MATLAB. Due to the extra space taken up by the access transistors in n-well sensors, a bead located in between two of these sensors on the horizontal line H-H' in Figure 4-11 registers a total signal that is approximately half of the signal from a bead located in the center of a sensor (Figure 4-12(a)). This smaller signal occurs because the bead is further away from each sensor and B_{bead} substantially decreases with distance as shown previously in Eq. (11) and Figure 4-4. In contrast, MOS Hall sensors do not require extra access transistors, resulting in a smaller pixel and a signal variation of 13%. On the vertical line V-V', there is significantly more signal overlap between adjacent sensors because as the bead gets closer to the polarization wire it becomes more strongly magnetized (Figure 4-12(b)). Although B_{pol} is hori-

zontal right above the wire, B_{bead} still has a substantial vertical component in the plane of the Hall sensor. Thus, as the bead is scanned across the V-V' line, the increasing B_{pol} counteracts the signal attenuation caused by the increasing distance between a bead and a Hall sensor, resulting in a nearly constant total bead signal across the V-V' line for both sensor implementations. The MOS sensor has a slightly skewed response on the V-V' line due to the off-center contacts.

Figure 4-12: (a) Normalized signal as a function of the position of one magnetic bead over the n-well sensor (left) and MOS sensor (right) on horizontal line H-H', shown in Figure 4-11. (b) Normalized signal as a function of the position of one magnetic bead over the n-well sensor (left) and MOS sensor (right) on vertical line V-V', shown in Figure 4-11. The total signal is the sum of the signals of the center sensor and adjacent sensors. The points and error bars show measured results.

Error bars in Figure 4-12 (a) and (b) represent the one-sigma signal variation among several measurements of bead signals at different locations of a sensor cell. Measured and simulated signals are in excellent agreement for both sensor implementations. Consequently, the simulator was used to calculate σ_{pol} to obtain $\sigma_{pol} = 0.13$ for the n-well sensor and $\sigma_{pol} = 0.06$ for the MOS sensor. According to Eq. (8), since both values are well below the Poisson error of $\sigma_P = 1$, they negligibly decrease the detector accuracy. This has two implications. First, there is room to make the sensor cell larger by, for example, increasing the widths of the polarization lines to carry more current and thus generate more signal without a substantial increase in total error. Second, it is now clear that the n-well implementation, due to its better time and energy metrics, is preferred; the n-well implementation was used in the sensor arrays that are discussed in chapter 5.

To verify that these arrays are capable of quantifying the total number of beads on the array, a known number of beads, counted under a microscope, were placed over the array and the sum of the signals from all Hall sensors in the array was obtained. This measurement was performed for several bead types including the 4.5 μm M450 beads, the 2.8 μm M280 beads, and the 1 μm MyOne beads. Figure 4-13(a) shows the total Hall voltage vs. the number of beads placed on the array. As expected, M450 beads have the largest response since they contain the most iron oxide per bead, whereas the MyOne beads have the smallest response. A linear fit is superimposed over the signal samples and the correlation coefficient was found to be $R > 0.997$, demonstrating that an array of Hall sensor bead detectors are capable of linear quantification of the number of beads on the array.

Looking back at Eq. (8), the required detection area A is proportional $d^2 \times n$, which is essentially the “area” of the beads on the array as seen from above the array. In other words, in measuring the performance of a particular bead using the framework presented in chapter 2, it is not the number of beads on the array that is important but the percentage coverage of the array by the beads. Beads that generate the highest signal per percentage of array area covered are preferable from a detector system performance point of view. In Figure 4-13(b), the total signal is plotted vs. the bead percentage area coverage. As in Figure 4-13(a) the M450 beads perform best since they are the largest and have the most iron oxide per volume. However, unlike in Figure 4-13(a), the MyOne beads have only a 20% smaller signal per bead area coverage than the M450 beads because they have the most magnetic content per volume (26% vs. 20% and 14% for M450 and M280 beads, respectively) and because their bead centers are closer to the sensors. The M280 beads have approximately 50% of the signal per bead area coverage as compared to the M450 beads because of their low iron oxide content.

Finally, in order to demonstrate the efficiency of placing bead centers closer to the Hall sensors, we plot the total Hall signal vs. the total volume of iron oxides (ferrites) on the surface in Figure 4-13(c). As expected, the smaller beads have a larger signal per iron oxide volume since they are, on average, closer to the surface than the larger beads.

Figure 4-13: Total voltage signal from all sensors in the array for three different bead types vs. (a) number of beads on the array, (b) percent of array area the beads cover, and (c) total volume of iron oxides (ferrites) from the beads on the surface of the array.

Figure 4-14 shows a “magnetic image” of a measurement similar to those in Figure 4-13 using both n-well and MOS Hall sensor arrays, alongside an optical image viewed

under a microscope with 400x magnification. The coloring corresponds to the amplitude of the Hall voltage signal for each sensor in units of bead signals, where one bead signal corresponds to the equivalent signal of a bead in the center of a sensor. Unlike other magnetic bead sensors such as GMR sensors and inductors [17], [27], which currently perform “bulk” detection of multiple magnetic beads, this platform is able to resolve individual beads anywhere within the array. As will be demonstrated in chapter 5, this property can be leveraged to detect a small number of beads in very large areas while maintaining sufficient quantification accuracy. The fine resolution may be further exploited to measure bead distribution and detect anomalies, for example in the case where non-Poisson distributions or large clumps of multiple beads occur, which may distort assay signals and are largely undetectable in “bulk” bead detectors.

Figure 4-14: Optical vs. magnetic images of magnetic beads on n-well and MOS Hall sensor arrays.

In order to characterize the thermal effects occurring in Hall sensors, an experiment was performed where all sensors in the array were measured and the pre-CDS signal was recorded. Figure 4-15(a) shows the results of the measurements of 4 corner sensors and one center sensor, showing the measured pre-CDS signal in the same form as discussed

in Figure 4-10. The current I_{pol} was chosen to be 32 mA for this experiment and the frequency was 62.5 kHz (about 4 μ s per phase as shown in Figure 4-9). On the left side of the array, temperature is increasing to the right (relative to Figure 4-15(a)) and the thermal gradient reduces the potential difference across Hall sensors contacts; hence we see what appears as an overshoot in the sensor signal when the polarization line turns and heats up the sensors. Conversely, on the right side of the array the temperature increases to the left (Figure 4-15(a)) and the thermal gradient increases the potential difference; hence the sensor signal appears to undershoot as the polarization line heats up the sensors. In the middle of the array the gradients are significantly smaller so the signal looks closer to an ideal square wave.

To verify that the non-ideal effects presented above are actually due to thermal effects, the period of each phase (i.e., frequency of excitation) and the polarization current were varied and the results are shown in Figure 4-15(b). As expected, at lower polarization current there is less heating and consequently the gradients are smaller and the input referred thermal error is smaller. In addition, at frequencies below the thermal time constant of the polarization wire / silicon system (which was calculated to be approximately 2 μ s and verified in the measurement of Figure 4-15(a)), the error due to thermal effects is substantially smaller.

Figure 4-15: (a) Pre-CDS response of Hall sensors at different regions in the 8x8 array. The gray squares represent dummy sensors and the white squares represent active sensors, (b) graph showing maximum error due to thermal effects for 6 mA and 25 mA I_{pol} vs. excitation frequency of the polarization line.

In order to assess the robustness of CMOS-integrated Hall sensors, a stress test experiment was performed. One row of sensors was selected to be measured four times over a period of 90 minutes. Between each measurement, the packaged chip was removed from the connector on the PCB, rinsed with water, heated to over 70°C for 20 s to remove the

water, and allowed to cool for one minute before being placed back on the PCB and measured again. Figure 4-16 shows the results of the measurements where each set of points connected by a line corresponds to one of eight sensors in the row. Although some small variations occurred in between the measurements, no drift was detectable on average. Sensor-to-sensor variations were measured to be 0.4% of the mean value, which contributes a negligible error to the bead signal compared to the other errors discussed in chapter 2.

Figure 4-16: Measurements of signal amplitude from one row of sensors over a time span of 90 minutes.

4.7 Summary

Hall-effect bead detectors were designed and characterized. The detector architecture is organized into an array of unit cells each containing a Hall sensor, bias and signal wires, and polarization wires to polarize the paramagnetic beads. The design includes a post-processing step to remove most of the inter-layer dielectric above the silicon in order to bring the beads closer to the sensors. Due to many non-idealities associated with CMOS-based Hall sensors and polarization wires, a correlated double sampling measurement scheme based on relaxation detection was developed to reliably extract the bead signal.

To validate the proposed bead detector architecture, measurement scheme, and to chose an appropriate type of Hall sensor, small arrays of n-well based and MOS transistor based Hall sensors were fabricated and characterized. Due to their superior sensitivity, the n-well sensors were chosen for further consideration and analysis. These sensors show a linear relationship between the number of beads and total signal and are able to resolve individual beads in the array. Further, the sensors show very little sensor-to-sensor variation and display no significant drift after being thermally and mechanically stressed.

5 Bead detector array

This chapter extends the bead detector design of chapter 4 to larger arrays derived with the design framework presented in chapter 2. Considerations for optimizing the performance of the bead array are first presented. Subsequently, the design of the readout circuitry is explained. The chapter concludes with an implementation and characterization of a 10,240 sensor array that can detect magnetic bead labels with sufficient accuracy and dynamic range for use in diagnostic applications.

5.1 Array design and optimization

Figure 5-1 shows the architecture of the Hall sensor array implemented in this chapter. Switches at the sides of the array connect one row of sensors to the supply lines ($V_{dd} = 2\text{ V}$ and ground = 0 V) while another set of switches (not shown) turn on the current in the polarization wires next to the enabled sensors to magnetize beads in their vicinity. The supply lines and polarization lines are shared between adjacent rows to minimize IR drop and increase sensor packing density. The word line is enabled to transfer sensor signal to the bit-lines that carry the signals to the top and bottom of the array to be digitized in parallel by a series of pitch-matched amplifiers and digitizers [35]. The array can be trivially scaled by utilizing multiple array banks or by adding more rows to the array (up to a limit of 64 due to thermal noise coming from the high resistance poly bit lines). The main design challenge thus lies in designing the individual row of sensors that makes up the array.

Figure 5-1: Array architecture.

In the array that was implemented the sensor size was selected to be $W = 3 \mu\text{m}$ and $L = 4 \mu\text{m}$ instead of $4 \mu\text{m}$ by $4 \mu\text{m}$ since this results in less power consumption by each individual sensor while maintaining approximately the same SNR [37]. The two main degrees of freedom that remain are the number of sensors per row, N_{row} , and the polarization current, I_{pol} . As N_{row} is made larger, more sensors are polarized and read out in parallel and the detector array is faster and more energy efficient. Unfortunately, metal lines have finite resistivity, so as N_{row} is made larger, the widths of polarization lines and the supply lines need to be scaled to maintain a suitably low voltage drop. Wide lines effectively add area overhead to the sensor unit-cell by creating regions in the unit-cell where beads are far away from the sensors, resulting in a small signal in those regions. Consequently, the mean bead signal drops and bead location error σ_{pos} increases, eventually decreasing overall system performance despite the extra parallelization benefit.

Consider next the choice of the polarization current I_{pol} . The detector total current consumption can be written as:

$$I_{dd} = I_{pol} + I_{sens} + I_{readout} \quad (19)$$

where I_{sens} is the current due to the Hall sensors and $I_{readout}$ is the current due to the readout circuitry. In most cases, doubling I_{pol} doubles the bead signal and therefore re-

duces measurement time by a factor of four in the case thermal noise dominates, which is true for detectors presented here. At the same time, power consumption increases by less than two times and therefore energy consumption is reduced by more than two fold. Thus, to first order, increasing I_{pol} results in a lower energy consumption and faster detection. The maximum I_{pol} that may be used is nevertheless limited by three effects:

First, as in the case of increasing N_{row} , the polarization wire width must be increased with increasing I_{pol} , eventually leading to reduced performance for the same reasons as described above for scaling N_{row} .

Second, there is a critical magnetic field B_{crit} , such that for fields larger than B_{crit} , the relationship between polarization field and bead magnetic moment becomes substantially non-linear and the bead signal begins to saturate. Increasing the polarization field beyond B_{crit} provides only incremental improvements in the signal strength and eventually degrades energy consumption. B_{crit} was measured for several beads and is on the order of 3-10 mT [25], [29], which roughly corresponds to I_{pol} values of 64 mA to 256 mA in the sensor arrays implemented in this work.

Third, device heating can become significant with increasing current. The total temperature at the surface of the chip comes from global heating of the entire system (i.e., chip, fluid, and printed-circuit-board) over the time a measurement is performed and from local heating of the chip surface above the sensors. Thermal simulations show that with a two layer PCB and proper design for heat spreading the global heating of the chip can be kept substantially lower than local heating of the chip surface. The relatively high local heating comes from the polarization wires, which carry large currents and are not connected (i.e., not thermally grounded) to the silicon substrate at the any point in the sensor array. Proteins and protein-to-protein bonds degrade rapidly at temperatures above 60°C-70°C [39], [40]. Although detection takes place after the other assay steps are already performed, total surface heating over 20°C-30°C may denature antibodies in seconds and may substantially weaken antibody-antigen binding forces, potentially breaking specific bonds and causing beads to move away from the sensors to corrupt the final signal.

To find the optimal sensor array structure, the array was simulated for a range of values N_{row} and I_{pol} to obtain the detection time, energy dissipated, and detection area for each array instance. Effects of magnetic field saturation were ignored for this simulation and were found to be negligible for optimal I_{pol} . For this particular example, a Poisson CV of $CV_P = 5\%$ was chosen along with a dynamic range of $DR_{label} = 100$, which at the lowest detectable concentration, corresponds to 400 beads covering 1% of the array. The mean signal and label position error σ_{pos} were calculated using the FEM simulator introduced in chapter 4. Total time was computed such that the total thermal noise CV was set equal to the Poisson error CV (i.e., $CV_{noise} = CV_P = 5\%$), which yields a final CV_{label} of 7.4%. Energy was computed by taking the product of the time and the average power dissipated. Finally, since time, energy, and area are important parameters for a POC system, the figure-of-merit (FOM) was chosen to be:

$$FOM \propto 1/(time \times energy \times area) \quad (20)$$

Figure 5-2 shows a graph of the FOM (black contours) for different array instances having different values of I_{pol} and N_{row} . The optimal range lies for I_{pol} between 128 mA and 512 mA and N_{row} between 16 and 48. At low I_{pol} and N_{row} , the signal is small and the array does not take advantage of parallelization enough. At high I_{pol} and N_{row} , the wiring overhead is so large that the average signal drops and position-dependent error increases beyond the Poisson error, reducing the performance.

Figure 5-2: Contour plots of FOM and maximum surface temperature rise for various values of polarization current and number of sensors per row.

The optimal currents suggested above are relatively high and cause substantial surface temperature rise. In order to quantitatively characterize the surface heating, thermal FEM simulations were performed by modeling the sensor row as an infinitely long structure as was shown previously in Figure 4-8(a). The maximum surface temperature is overlaid on Figure 5-2 (red contours). In general, the more current is used the more heat

is generated, whereas the smaller the N_{row} is made the narrower the polarization lines become and the less efficiently the heat is transferred to the silicon substrate, resulting in a larger temperature rise. The simulation shows that at $I_{pol} = 128$ mA and $N_{row} = 32$ the FOM is within 30% of the maximum value and heating is below 10°C, yielding a good safety margin from a maximum rise of 20°C–30°C, and hence this is the preferred point of operation.

A large Hall-effect sensor array was designed and implemented for quantifying microbeads on its surface with high accuracy. The detection area was chosen based on Eq. (8) for 2.8 μm beads, a DR_{label} of 100, and CV_{label} of 5%, which, for a power law relationship of Eq. (2) with $x = 2$, gives assay parameters of $DR_{[A]} = 10,000$ and $CV_{label} = 10\%$. A minimum number of $n = 400$ beads is required to overcome Poisson error and a maximum number of $n \times DR_{label} = 40,000$ beads needs to fit within the detection area, which evaluates to 0.31 mm^2 . We designed and implemented an 8,192 sensor array that has a detection area of 0.52 mm^2 to meet these specifications. In order to ensure robust operation, this version of the array was designed for a current $I_{pol} = 32$ mA array and has 32 active sensors per row ($N_{row} = 32$). The array consists of 4 banks of 2048 active sensors each and is shown in Figure 5-3.

Figure 5-3: (a) Optical image of the sensor chip containing 4 banks with 2048 active sensors each. (b) One half of a 2048 Hall sensor array bank with M280 beads covering approximately 2% of the surface.

5.2 Bias and readout circuits

Figure 5-4 shows a high-level diagram of the circuit sub-system [34]. The chip is subdivided in two 4,096 sensor sub-arrays, each comprising a polarization current generator, a timing generation block, and two banks of 2,048 Hall sensors with associated readout electronics. When a row is selected, digital switches allow polarization current to flow

into one row of sensors in one of the sub-arrays, where it is switched from being routed to a row in the left bank or to a row in the right bank. When a row in one bank is in the relaxation phase, the corresponding row in the other bank is in polarization phase; thus the current is not wasted and the measurement time is cut in half. Each row of sensors is read out by a readout channel that includes circuitry implementing low noise amplification, averaging, and digitization. The layout of these circuits was designed to have a width equal to exactly two sensor pixels, allowing the electronics to be arrayed at the top and bottom periphery of the sensing area.

Figure 5-4: Array and electronics block diagram.

The readout channel used to achieve readout functionality is shown in Figure 5-5. It consists of an auto-zeroed voltage-to-current (V/I) converter that provides offset suppression and a current-mode first order incremental analog-to-digital (A/D) converter that provides digitization. The operation of the readout channel and the design of each sub-circuit are further described below.

Figure 5-5: Readout channel schematic.

As discussed in chapter 4, CDS is used to attenuate sensor offset, stray fields, $1/f$ noise, and thermal effects. A CDS offset cancellation scheme enabled by the readout channel is shown in more detail in Figure 5-6. It uses the same 4-phase non-overlapping clock used for enabling the polarization wires to implement noise-free auto-zeroing and boxcar sampling. Each of the four clock phases is denoted using the symbol Φ_X , where X is between 1 and 4. Each corresponding Φ_X' refers to the same clock phase but delayed by approximately 2 ns from the rising edge of Φ_X . This is to allow the magnetization signal to decay (with time constant $\tau_{LR} = 200$ ps) before a measurement is initiated in phases Φ_2 and Φ_4 . Other clocks used in the readout channel include a 1 MHz clock for the over-sampled ADC (signal Φ_{ADC}), a clock used read-out the decimated ADC output and operate the digital nested chopping loop (Φ_{CDS}), a clock used to reset the A/D loop filter (Φ_{RST}), and a clock for comparator sampling that occurs on the rising edge of signal Φ_c , which rises a short delay after Φ_1 .

The operation of the readout channel proceeds as follows. During phase Φ_1 , polarization field B_{pol} is applied to the sensor by driving current $+I_{pol}$ through the polarization lines. Concurrently, the Hall sensor produces an output consisting of its electrical DC offset and the aforementioned low frequency errors, the magnetization baseline, and the magnetization signal from any label present on the surface above the sensor. During this period, the V/I converter is disconnected from the A/D converter and is auto-zeroed, storing all the aforementioned error terms on the AC coupling capacitor C_{AC} .

During phase Φ_2 , the magnetization field is removed and the decay from the magnetic beads is measured by the Hall sensor, generating a voltage signal at the input of the V/I converter. The V/I converter is connected to the ADC through the direct path of the chopper $C1$ during Φ_2' . The signal appearing at the sensor output is then integrated on the ADC loop filter. In this phase, errors from the polarization baseline, thermoelectric ef-

fects, and circuit non-idealities such as finite gain (which reduces offset by 55 dB), charge injection, and sampling noise stored on C_{ac} by the auto-zeroing operation are integrated along with the signal. After integration the total signal error, V_{err} , is still 100–1000 times larger than the desired signal and must therefore be further suppressed.

Figure 5-6: V-I converter operation, from top to bottom Φ_1 (Auto-zero), Φ_2 (First integration), Φ_3 (idle) and Φ_4 (second integration)

The subsequent error suppression is achieved by repeating the measurement in Φ_3 and Φ_4 (and Φ_4') using opposite magnetization field polarity. To preserve errors unchanged

from Φ_1 , the amplifier is disconnected from the A/D during Φ_3 and the ac-coupling capacitors C_{ac} are not refreshed in this time. Instead, the V/I converter outputs are shorted together to prevent drifts and memory effects. At the same time, an inverted polarization field is applied by reversing the direction of current flow through the polarization wires. As a result, when in Φ_4 the V/I converter is connected again to the A/D loop filter, this time using the inverting path of chopper C1, the error sources described above are integrated a second time with opposite polarity, and hence to first order eliminated. In practice, the amount of suppression achievable is determined by the difference between the duration of Φ_2 and that of Φ_4 , which at 2 MHz can easily be less than 1000 ppm by careful clocking design. Since the magnetization field in Φ_3 is reversed, the signal from the bead is added constructively.

The schematic of the V/I converter is shown in Figure 5-7. A current reuse stage with both top and bottom current source bias is chosen to increase the g_m/I_d ratio and the noise efficiency while preserving supply noise rejection. The chosen transistor sizes result in a g_m/I_d ratio of 20 V^{-1} for the NMOS devices and 18 V^{-1} of the PMOS devices, leading to an overall $g_m/I_d = 38 \text{ V}^{-1}$. Common-mode feedback is applied through transistor M_3 and M_4 by degenerating the topside current source [41]. The non-linearity and differential-mode to common-mode cross talk of this architecture are avoided as the V/I converter output is connected to a differential-mode AC ground.

Figure 5-7: G_m -stage schematic with annotated transistor sizes

The area of input transistors is chosen based on a trade-off between area and noise efficiency. A large channel length for the input transistors reduces their $1/f$ noise and hence lowers the post-chopping white noise floor [42]. However, this demands both a larger area for the transistor, as well as a larger value and area for C_{ac} in order to prevent significant voltage loss from the capacitive divider between this capacitor and the parasitic at the gate in phases Φ_2 and Φ_4 . For the chosen sizing, the simulated $1/f$ corner of the amplifier is 830 kHz and the gate parasitic is approximately 100 fF. The capacitor C_{ac} is set to 1.1 pF and realized with a custom capacitor consisting of poly-poly cap stacked with custom M3-M5 finger capacitor to minimize area. The chopper is implemented with minimum-size transistors to minimize charge injection mismatch. The overall transconductance of this stage is 1.2 mS, while the loop filter integration capacitor C_{int} is set to 200 fF, obtaining a conversion gain to the integrator output of:

$$A = \frac{G_m}{C_{int}} \frac{T_m}{2} = 63.5dB \quad (21)$$

The factor of 2 in Eq. (21) is caused by the fact that the RMS value of the chopping waveform is $\frac{1}{2}$ and not 1 as in conventional chopping. In other words, the signal is observed and averaged over only 50% of the clock period. The simulated input-referred noise density of this V/I converter after chopping at 2 MHz ($T_m = 500$ ns) is 10.8 nV/Hz^{0.5} (216 nT/Hz^{0.5} after referring to the sensor input) while consuming 80 μ A from a 1.8 V supply. This corresponds to a noise-efficiency factor of 3.5 [43]. Finally note that the thermal noise from auto-zeroing in Φ_1 has a PSD given by:

$$N_{AZ} = 2 \frac{kT}{C_{ac}} T_m = 63nV/\sqrt{Hz} \quad (22)$$

Since this is much higher than the input referred noise of the V/I converter, it is vital to cancel this noise contribution through double integration as presented in Figure 5-6.

The current-input, continuous-time incremental A/D converter, which is clocked by the 1 MHz Φ_{ADC} signal, converts its average input current into a 10-bit digital output updated by the 1 kHz Φ_{RST} signal. The ADC outputs are further averaged in software to reduce noise. Additional details about the A/D architecture are provided below.

The A/D converter employs a first order, continuous-time incremental modulator with current input. A critical advantage of this architecture is that the integrator in the loop filter not only provides quantization-noise shaping, but it also accumulates the charge produced by the V/I converter during each 500 ns modulation period over the 1 ms ADC readout period, effectively reducing the input-referred noise. Since no separate charge accumulator is required, this reduces the interface component count, allowing a more compact implementation.

The ADC operates on a 2x divided clock compared to the V/I converter ($f_{ADC} = 1$ MHz) and produces an output digital word at 1 kS/s, providing a nominal 10-bit resolu-

tion. The converter offset, largely determined by the OTA used in the loop filter, can be as large as 500 μT when referred to the input of the Hall sensor. The ADC also limits the residual system input-referred flicker noise corner to 1 kHz. This offset and its drift are suppressed by a nested chopping loop with digital demodulation ([41], [44]) that operates by exchanging the sign of the magnetization field in Φ_1 and Φ_3 after each A/D sample is taken, effectively up-modulating the bead signal with a 1 kS/s square wave. The signal is demodulated subtracting consecutive A/D output words in the digital domain, removing residuals $1/f$ noise and offset, including the contribution of the converter itself. The field polarity is switched in a silent clock phase (Φ_{RST}), which is also allocated to resetting the integration capacitor. As a result, to first order no nested chopping transients are observed. Because the offset is suppressed only after digitization, the A/D converter is designed to have a ± 2.5 mT full scale to prevent residual offsets from saturating this block prior to demodulation. The corresponding quantization noise has an input-referred PSD of $52 \text{ nT/Hz}^{0.5}$, well below the V/I converter thermal noise.

Figure 5-8: Circuit Schematics of A/D Converter building blocks: D/A (left) and OTA (right)

The 1-bit D/A converter is realized with a complementary structure (Figure 5-8), where both the top and the bottom current sources are switched, leading to a 2x reduction of thermal noise. The OTA used in the integrator uses a folded-cascode topology with extra-cascode devices in the input path to provide 75 dB of DC gain and ensure effective averaging over 1000 samples. Its current consumption is set to 80 μA to provide low effective input impedance to the V/I converter, thus minimizing signal-dependent charge injection from chopper C1 without resorting to a two-stage design. Common-mode feedback is realized with a switched-capacitor network clocked at 250 kHz. A 14-bit counter and shift register provides decimation and data output serialization.

5.3 Measurement results

5.3.1 Sensor characterization

In addition to the large array having $I_{pol} = 32$ mA and $N_{row} = 32$, a high performance 8×8 test array designed for $I_{pol} = 128$ mA and $N_{row} = 32$ was implemented. To characterize the sensors, each array implementation was tested using $4.5 \mu\text{m}$ M450® beads as they can be positioned accurately using a micromanipulator. Table 5-1 lists several parameters and measurement results obtained for each unit cell. The 128 mA sensors were measured up to 106 mA due to current routing limitations. Since no degradation of signal due to bead signal saturation or temperature rise was seen, the results of the 106 mA measurement were extrapolated to $I_{pol} = 128$ mA. The 128 mA polarization wires generate 3.2 times larger signals than those generated by the 32 mA polarization wires, mostly because their current density is further from the sensor/bead than the 32 mA polarization wires. Sensitivity was simulated across the sensor surface and the simulations were verified by measuring beads at the top, bottom, and corner of each unit-cell. Despite the substantially wider polarization lines needed to deliver a 128 mA polarization current, bead signals are still relatively uniform within the 128 mA sensor unit cell with a $\sigma_{pos} = 0.20$ for $4.5 \mu\text{m}$ beads and $\sigma_{pos} = 0.47$ for $1 \mu\text{m}$ beads. Even in the worst case of $\sigma_{pos} = 0.47$, a 5% CV_{label} due to Poisson error would be increased by $(1^2 + 0.47^2)^{1/2}$ to get a CV_{label} of 5.5%, which is a relatively small increase in error.

Sensitivity variations between different sensors in the array were measured to have a standard deviation of 1.4%, adding negligible signal error. To evaluate the variations in beads (σ_{sig}), signals from several individual $2.8 \mu\text{m}$ and $4.5 \mu\text{m}$ beads were measured and the standard deviation was computed to be $\sigma_{sig} = 0.096$ for $4.5 \mu\text{m}$ beads and $\sigma_{sig} = 0.31$ for $2.8 \mu\text{m}$, in both cases much smaller than the Poisson error, indicating that variations in these labels do not substantially degrade accuracy.

Parameter	32 mA sensor	128 mA sensor
Polarization current I_{pol} (mA)	32	128
Width of polarization lines (μm)	1.6	6
Width of supply lines (μm)	1	1
Unit-cell dimensions, width by height (μm)	8 by 7.9	8 by 12.3
Label pos. error σ_{pos} for 4.5 μm beads	0.13	0.20
Label pos. error σ_{pos} for 1 μm beads	0.34	0.47
Max. vertical polarization field (mT)	3	9.6
Max. field from 4.5 μm bead (μT)	17	53
Sensitivity (V/V/T)	0.025	0.025
Hall voltage from 4.5 μm bead (μV)	0.85	2.7
Optical picture and sensitivity heat map

Table 5-1: Summary of sensor parameters for 32 mA sensors and 128 mA sensors for detecting 4.5 μm beads. Optical images and sensitivity heat maps for each implementation are also shown.

5.3.2 Array characterization

For the sensor array to function as bead detector, it must display a known and preferably linear transfer function between the number of beads on the array and the sum of the signals of all sensors in the array. Figure 5-9 shows the relationship between the number of beads placed on the array vs. the total relaxation signal obtained from the sensors in the array for three different beads, 4.5 μm M450, 2.8 μm M280, and 1 μm MyOne beads. Two of the array banks and a total of 4,096 sensors, spanning approximately 0.26 mm^2 , were used to perform this measurement. The response is linear with $R > 0.99$ for all three bead types, and one 4.5 μm bead produces an 8 times and 20 times greater signal than one 2.8 μm and one 1 μm bead, respectively.

Figure 5-9: Sum of all bead signals from all sensors vs. number of beads placed on the array

To test the accuracy of the array, 440 M280 beads covering approximately 1% of the array, close to the worst-case minimum of $n = 400$, were placed on the array and measured. Using a measurement time of 480 ms per sensor row and 30 s for the entire array, the error due to thermal noise has a CV of 5.3%, which is similar to the Poisson error for $n = 440$. Figure 5-10 depicts errors from various sources, including the expected Poisson error and the measured thermal noise, as a function of the number of beads on the array. Measured values are indicated by points, and for $n = 440$ and a measurement time of 30 s, the total error has a CV of 7.4%. Only 0.3% of the 7.4% error is contributed from bead variations σ_{sig} and from bead position variations σ_{pos} .

Figure 5-10: Coefficient of variation vs. number of beads placed on the array for various error sources. Points indicate measured results and lines indicate simulated or calculated values

Based on the measured signal values for each of the beads and the measured thermal noise of the array, the detection time, energy, and the required area to achieve a $CV_P = CV_{noise} = 5\% \Rightarrow CV_{label} = 7.1\%$ and $DR_{label} = 100$ are derived for M450, M280, and MyOne beads and shown in Table 5-2. Larger beads require larger areas but generate larger signals, thus having a shorter measurement time. For all the beads the detection times are below one minute and the energy requirements are well below those supplied by small batteries. Interestingly, MyOne beads have the highest FOM for two reasons: (1) they require a comparably small detection area due to their smaller size while generating a relatively large signal due to a high magnetic content, and (2) the fact that their bead centers are closer to the Hall sensors than those of the larger beads. This is consistent with the measurements of the test array in Figure 4-13(b), except since area is also a factor in the FOM calculation, the MyOne beads outperform the M450 beads.

Bead	Area	Time	Energy	FOM
M450	0.81 mm ²	8.5 s	1.3 J	320
M280	0.34 mm ²	40 s	6.2 J	35
MyOne	0.045 mm ²	13 s	2.1 J	2,500

Table 5-2: (a) Time, energy, required detection area, and FOM for various bead sized required to obtain a $CV_{\text{label}} = 7.1\%$ and $DR = 100$ for a Hall sensor array implementation with $I_{\text{pol}} = 32$ mA and $N_{\text{row}} = 32$.

Figure 5-11 shows the detection time vs. bead size of beads measured in Figure 5-9 for a $CV_{\text{label}} = 5\%$ and a $DR_{\text{label}} = 100$, equivalent to 400 beads covering 1% of a sensor array. The detection time is simulated and extrapolated to smaller beads based on the relative signal of the MyOne bead, accounting for smaller or larger size using our FEM simulator. For the array architecture having $I_{\text{pol}} = 32$ mA and $N_{\text{row}} = 32$, magnetic beads having a diameter of 500 nm can be accurately detected in less than one minute. The results are further extrapolated to an architecture based on 128 mA polarization currents characterized in Table 5-1. Using the 128 mA architecture, microbeads can be quantified within 5 seconds and 200 nm beads can be accurately quantified in less than one minute. Beads larger than 4.5 μm provide little signal gain since their centers become significantly far away from the polarization lines and Hall sensors. As expected, the 2.8 μm beads are far from the extrapolated curve due to their relatively low ferrite content.

Figure 5-11: Required detection time to achieve a CV_{label} of 7.4% and DR_{label} of 100 vs. bead diameter for sensor based on the 32 mA and 128 mA polarization currents.

5.3.3 Signal demodulation using thresholding

In the analyses and measurements presented above, the total array signal is obtained by simply summing the signals from the individual Hall sensors. This method has a drawback in that uncorrelated thermal noise from all the sensors in the array is also added together, doubling the total noise voltage every time the array is made four times larger. Measurement times for each individual sensor can be increased to counter this effect, but this increases the total detection time, which as discussed in chapter 2 is limited to one minute for POC applications. Because of this addition of noise, the dynamic range for microbead labels on the Hall sensor array presented here is limited to about $DR_{label} = 100 - 300$, corresponding to 0.3% - 1% bead area coverage. While this range is sufficient for many assay applications, in some assays, especially those where there is a linear or nearly linear relationship between the number of magnetic labels and the analyte concentration [10], a larger dynamic range is needed.

Another method for demodulating the signal from the sensors to a final resulting signal can be used with this architecture. Since microbeads generate relatively large, spatial-

ly concentrated signals, a single bead positioned over a $3\ \mu\text{m}$ by $4\ \mu\text{m}$ Hall sensor can be detected with an SNR of 20 dB in less than 100 ms [33]. Further, since at low bead concentrations there are statistically either zero beads or one bead over a sensor (rarely are there two or more beads), a single threshold level can be used per sensor to decide whether a bead lies above the sensor or not without losing any information. At SNR of 20 dB, false positive and false negative decisions for beads positioned in the center of a sensor occur with a probability less than 1 in 1 million, enabling very large dynamic ranges. These individual decisions can then be added up in the digital domain without adding up noise.

Despite the clear advantages, thresholding demodulation has several drawbacks specific to Hall sensor arrays. First, bead signals vary with position over a sensor and no matter what value a threshold is set to, a bead may land in a position where its signal is close to the threshold, producing a large variance in decisions in the presence of thermal noise. Second, beads landing in between two sensors, which are still partially counted using summing demodulation, will not be detected for thresholding demodulation using reasonable thresholds, effectively decreasing the total array signal and increasing position dependent error σ_{pos} .

To quantify the performance of thresholding demodulation vs. summing demodulation, an experiment using 13 M450 beads placed on the 4,096 sensor array was performed. The optimal threshold was chosen empirically and via statistical simulations, which showed an excellent match. Each sensor was measured for 35 ms, 70 ms, 140 ms, and 1120 ms, generating an SNR per bead of 14 dB, 17 dB, 20 dB, and 29 dB, respectively. A CV_{noise} of 15% was achieved for the thresholding demodulation for the 20 dB SNR measurement whereas as for summing demodulation the CV_{noise} was measured to be 55%. To understand how thresholding modulation allows for scaling an array to a large number of sensors, the results of the experiment are presented in Figure 5-12 where the horizontal axis is the number of sensors and the vertical axis is the required measurement time to obtain a CV_{noise} of 15%. To measure effectively less than the 4,096 sensors of the array, for each measurement the sensors under and in the vicinity of the 13 beads were measured and a variable amount of additional sensors were also measured, simulating a variable size array. As more sensors are added, more noise is added and more time is required for summing demodulation to obtain a constant CV_{noise} . For thresholding, the required time also increases but the increase is much shallower as bit errors become extremely rare for high SNR values. The 13 beads, covering only 0.1% area of the array, are detected in 9 s total. Extrapolating this result shows that an array with 30,000 sensors can readout 13 beads in 60 s with a CV_{noise} of 15% (assuming $N_{row} = 32$), showing that the thresholding technique can achieve dynamic ranges in excess of $DR_{label} = 5,000$.

Figure 5-12: Measurement time per sensor required to quantify 13 beads with a CV_{noise} of 15% vs. number of sensors in the array

5.4 Assay results

To verify the functionality of the sensor array in an assay context, a reverse phase direct assay for Human Serum Albumin (HSA) was performed. The assay used anti-HSA antibody coated M280 beads as labels. The sensor surface was first functionalized with a known concentration of HSA using physical adsorption. After blocking the surface with 1% Bovine Serum Albumin (BSA), the magnetic beads were added and incubated for 2 minutes. After incubation, non-specifically bound beads were removed with external neodymium magnets. The measured output from the magnetic sensor is shown as a function of HSA concentration in Figure 5-13. The figure also shows optical images of the sensor array after the washing step for each of the concentrations reported. The assay has a detection limit of 100ng/ml. No signal is seen for the 1ng/ml case likely due to excessive washing forces used in this experiment.

Figure 5-13: HSA assay using 2.8μm labels: normalized signal output vs. HSA concentration. Optical photos of the measured sensor array are also shown for each measured concentration.

To extrapolate the performance of the sensor array to a sample magnetic bead assay in literature, we can use the results from the assay in [12] which was performed on a glass substrate. The paper reports that for Prostate Specific Antigen (PSA), a prostate cancer marker, the relationship between specifically bound beads and the PSA concentration, normalized to the detection area of the chip used in this thesis, is:

$$\log n = 0.35 \log([PSA]) + 2.41 \quad (23)$$

where [PSA] is the concentration of PSA expressed in ng/ml and n is the number of 2.8 μm magnetic beads. Using a Poisson CV of $CV_P = 5\%$, which corresponds to $n = 400$ beads, yields a detectable concentration of PSA of [PSA] = 3.5 ng/ml. Assuming Poisson error is dominant, the assay CV is $CV_{[PSA]} = CV_P / 0.35 = 14.3\%$, which translates to a standard deviation in the concentration of 500 pg/ml. On the other hand, with an allowable CV_P of 10% (which leads to a $CV_{[PSA]}$ of 28.6%), the detectable concentration of PSA falls below 100 pg/ml, more than 30x lower. These metrics are sufficient for high accuracy cancer screening, where PSA concentration values below 4ng/ml are considered normal [45].

6 Conclusion

Magnetic beads are excellent candidates for labels in biological assays performed on integrated platforms. In this work we have demonstrated that magnetic beads on the order of $1\text{ }\mu\text{m}$ in diameter can be quantified with accuracies sufficient for biological assays in detection areas small enough to fit on CMOS chips and with a detection time and energy suitable for POC settings. Magnetic bead detectors based on n-well Hall sensors and polarization wires were designed in a $0.18\text{ }\mu\text{m}$ CMOS process and display a good combination of sensitivity and robustness. Relaxation detection and one-point calibration techniques were applied to further increase the accuracy of bead detection. The individual sensors were organized into a $4,096$ sensor, 0.26 mm^2 array that can quantify a 1% surface coverage of magnetic microbeads with a worst case CV of 7.4% within one minute while dissipating less than 10 J of energy, less than 1/2000 of the energy stored on a typical smartphone battery. Utilizing thresholding demodulation, a feature of this implementation due to the inherent array-based design, beads covering only 0.1% of the sensor array can be detected in just a few seconds with high accuracy. The microchip was tested in a biological setting and is capable of detecting beads in biological conditions with protein concentrations down to the 100pg/ml levels. To conclude, robust and compact detection platforms based on CMOS Hall-effect sensors and magnetic microbead labels are suitable for high accuracy point-of-care assays.

7 References

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