Fully Integrated Complementary Metal Oxide Semiconductor (CMOS) Bio-Assay Platform



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Fully Integrated Complementary Metal Oxide Semiconductor (CMOS) Bio-Assay Platform

by

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requirements for the degree of

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Abstract

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Professor Bernhard Boser, Chair

We present a post-processed $6.25 \text{mm}^2 0.18 \mu\text{m}$ Complementary Metal Oxide Semiconductor (CMOS) platform that leverages the advantages of super-paramagnetic bead labeling to integrate on-chip the label separation and detection functionalities required for high sensitivity bio-assays. The surfaces of the CMOS chip and of the magnetic beads are functionalized with bio-chemicals complementary to a target analyte. In a sandwich capture format, the presence of the target analyte will strongly bind 4.5 μ m magnetic bead labels to the surface of the chip. The undesired background signal is minimized by the removal of the unbound magnetic beads from the detection array via magnetic forces generated on-chip. The remaining strongly bound magnetic beads are respectively magnetized and detected by an array of 128 stacked micro-coil/Hall sensor elements. This single chip solution does not require any external components like pumps, valves or electromagnets and is capable of detecting purified Human antibodies down to concentrations of 100pg/ml as well as anti-Dengue antibodies in human serum samples.

A whole blood sample preparation system based on membrane filtration alleviates the need for centrifugation and can be readily combined with the assay platform into a high performance, Point-of-Need (PON) *In-Vitro* Diagnostic (IVD) device.

The present prototype relies on an electronic reader for controlling the assay and reporting results. Implementing this function on the bio-sensor front-end enables a very simple design consisting of only the bio-sensor and an attached results display. Such lowcost, easy-to-use, high performance devices are needed for lowering health costs through more decentralized distribution of medical care. To my family, Eugen, Viorica, Valentin and Iky.

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Chapter 1

1 Point-of-Need In-Vitro Diagnostics

Octavian Florescu

1.1 Introduction

The last two major technological revolutions, namely the automotive and the Information Technology (IT) revolutions, made accessible directly at the Point-of-Need (PON) products and services that were previously only available through centralized distribution. In addition to significant reduction in the opportunity costs of transportation and information, the added user flexibility in combination with the requisite individual freedom led to the countless innovations that are now bedrocks of our modern industrial societies and that contribute greatly to the general state of welfare.

The healthcare sector, on the other hand, has retained privately and publicly accredited centralized sources of distribution. This structural rigidity manifests itself as rising healthcare costs relative to general consumer costs, since the latter benefit from more unfettered competition and fluid re-organization. Medical technologies targeted to the PON have the potential to circumvent a number of market barriers, thereby releasing economic efficiencies locked into the healthcare sector. Many medical diagnoses and therapeutics could be delivered much like food, another vital necessity whose quality is regulated by the federal government, but whose cost has been declining relative to wages.

To ensure widespread adoption, PON medical devices must be easy to use by the general public and have a low total system cost, in addition to meeting the usual standards of efficacy placed on all medical devices.

Soluble bio-marker detection is particularly well-suited for the PON since a wide range of medical conditions from cardiac disease to cancer to communicable infections can be identified using *In-Vitro* Diagnostics (IVD). Moreover, identification or quantitation of bio-markers like anti-HIV antibodies can result in timely, actionable information.

The identification of soluble bio-markers from raw samples often begins with labeling the target molecule using fluorophores, enzymes or magnetic particles. For high sensitivity applications like for the early detection of infectious diseases, the target molecule bound labels must be discriminated from the unbound ones that contribute to background noise. Finally, the bound labels are detected and quantified if necessary.

1.2 Immuno-Chromatographic Tests

By combining label separation and detection, the Immuno-Chromatography Test (ICT) has become the dominant technology for low cost, easy-to-use, PON IVD. Its operation, presented in Figure 1.1, begins with the wicking into the large pore size sample pad of a raw sample containing the target analyte. Capillary forces pull the sample through the conjugation pad where lyophilized labels, such as colloidal gold or latex particles, fluorophores, etc, resolubilize and bind to a first epitope on the soluble analyte. These labels are then pulled further along the nitrocellulose strip until reaching the test line coated with lyophilized protein complementary to a second epitope. The presence of the analyte will separate the bound labels that become tethered to the test line from unbound labels that continue down the strip due to capillary forces, eventually binding to the control line for a valid test. As the normally invisible labels aggregate onto the lines, they become visible to the unaided eye. Both the control and the test line must be visible for a valid positive result, while only the control line must be visible for a valid negative result.

Despite their commercial success, ICTs suffer from low biochemical sensitivity, ambiguous reporting, limited quantitation, timing requirements, and awkward multiplexing, and therefore cannot be used for a range of bio-marker applications¹. The bio-chemical sensitivity of ICTs is limited to approximately 35pM on purified protein² to 1.6nM on raw samples³.



Figure 1.1: Immuno-chromatographic test operation (Courtesy of NASA)

1.3 Integrated Digital Assay Technologies

Modern "lab-on-a-chip" technologies have shown the potential to overcome some or all of those drawbacks. In particular, micro-fluidic systems have been proposed as candidate technologies for PON IVD⁴. They consist of micron-scale channels patterned in plastic or glass that manipulate samples using automated protocols similar to the ones currently used in diagnostic laboratories. Unfortunately, actuation of the pumps and valves and the label detection often requires off-chip components, which makes the systems expensive, difficult to use and preclude stand-alone operation.

Attempts at integrating or even eliminating the off-chip components have been mixed. The Motorola effort saw a variety of electrochemical and thermopneumatic pumps and valves integrated into a single DNA biochip⁵. This blunt, head-on approach did not effectively collapse the protocol and as a results was plagued by prohibitive manufacturing costs.

Others have used Printed Circuit Board technology to electronically control the wetting characteristics of conductors used to guide and manipulate drops of fluid⁶. High sensitivity applications using this approach have been stifled by the daunting sample preparation hurdles associated with controlling the contact angle of complex physiological fluids.

In an acknowledgement to the inherent cost of conventional micro-fluidics, Sia et al. developed an automated immuno-assay using a single capillary that did not required any valves or pumps⁷. However, the performance of this system showed no improvement over conventional immuno-chromatographic tests, and cost significantly more due to the multi-chip implementation.

Most recently, Proctor & Gamble Co. has released the Clearblue Easy Digital Pregnancy Test onto the over-the-counter market⁸. This platform technology consists of an immuno-chromatographic strip mated to an inexpensive optical detector and a digital readout. While its commercial success is indicative of the demand for decentralized, consumer-driven health, the Clearblue easy platform has failed to demonstrate non-inferior performance over existing immuno-chromatographic tests; early reviews by consumers suggests an alarming rate of false positives compared to existing at-home pregnancy tests⁹. Moreover, the implementation consists of no less than 7 separate Integrated Circuits (ICs).

1.4 IC-Based Magnetic Bead Labeled Assays

By combining high specificity label separation, high sensitivity label detection and control electronics into an integrated system consisting solely of one chip, one battery and one display, IC based implementations have the potential to produce digital PON IVD platforms with low cost and high functionality,. In particular, single Complementary Metal Oxide Semiconductor (CMOS) chip designs have the potential to achieve

generational low cost structure, i.e. until the next generation low cost digital technology displaces CMOS.

The IC-based implementations proposed to date however require either off-chip components for label detection¹⁰, micro-fluidic actuation for label separation¹¹, or nonstandard processes for manufacturing^{10,12}.

We propose a fully integrated CMOS-based platform that can perform both label separation and detection without aid from off-chip components. The use of micron-sized magnetic beads as assay labels greatly facilitates protocol integration since these beads can be both detected and manipulated electromagnetically. Moreover, magnetic beads have additional qualities that make them ideal for PON application; a) the signals from magnetic beads are not affected by biological interactions and can be detected in opaque solutions like whole blood, b) the biological magnetic background signal is very low, c) the signals from magnetic beads are stable over time and insensitive to changes in temperature or chemistries, d) incubation with magnetic beads permits quasi-solution phase binding kinetics through their high surface area to volume ratio¹³, e) rapid sedimentation rates enables analyte concentration to the surface of the IC without relying on slow diffusion processes, and finally f) the ability to detect individual assay labels obviates the need for lengthy signal amplification.

The magnetic beads used for this work, shown in Figure 1.2, are spherical polymer matrices enclosing magnetite or maghemite nano-particles. For bio-assay applications, these beads must display super-paramagnetic behavior, i.e. they become magnetized only in the presence of an applied magnetic field. Otherwise, if they display ferromagnetic behavior and they retain their magnetization after removal of the applied field, the labels will aggregate in suspension and interfere with the assay protocol.



Figure 1.2: Magnetic bead labels

The particles' magnetic dipole moments m_p align more strongly with increasing magnitude of $B_{applied}$, until the magnetization of the bead M_{bead} saturates. This behavior is shown in Figure 1.3, and is described by a Langevin function¹⁴:

$$\mathbf{M}_{\text{bead}}(\mathbf{B}_{\text{applied}}) = M_{sat} \left[\coth\left(\frac{m_p B_{applied}}{kT}\right) - \left(\frac{kT}{m_p B_{applied}}\right) \right] \cdot \frac{\mathbf{B}_{\text{applied}}}{B_{applied}}$$
(1)

 M_{sat} is the saturation magnetization of the bead, k is the Boltzmann constant, T is the temperature, and μ_0 is the permeability of free space. In Figure 1.3, the measured magnetization per unit weight of a Dynal M-450 bead is plotted versus the applied magnetic field¹⁵. Note that the intersection of the curve with the origin implies superparamagnetic behavior. For small applied magnetic fields, such as those generated onchip, we can linearize about the origin and estimate:

$$\mathbf{M}_{\mathbf{bead}}(\mathbf{B}_{\mathbf{applied}}) = \frac{\chi_{bead} \mathbf{B}_{\mathbf{applied}}}{\mu_0}$$
(2)

 χ_{bead} is the susceptibility of the bead medium, which is 1.75 for the M-450 beads for a small applied magnetic field. This induced bead magnetization will be measured on-chip and used to detect the presence of a bead.



Figure 1.3: Dynal M-450 magnetization measurements¹⁵

Magnetic beads are also effective labels for manipulating bio-chemicals, and have long been used for separating and purifying target analytes from bulk mixtures. The general equation of the magnetic force resulting from a magnetic field is given by¹⁶:

$$F_{mag} = \frac{V_{bead} \chi_{bead}}{\mu_o} \left(\mathbf{B}_{applied} \cdot \nabla \right) \mathbf{B}_{applied}$$
(3)

As with all dipoles, the translational force is determined by the product of the applied field and its gradient. While on-chip generated magnetic fields are limited to the mT range, the field gradients at the micron scale are on the order of 100mT/m, resulting in forces on the order of 1pN.

The stage is therefore set for an IC platform that can integrate both separation and detection of magnetic beads in a fully integrated bio-assay platform.

1.5 Fully Integrated CMOS based bio-assay platform

For this work, CMOS was chosen over competing IC technologies because of its low cost and design versatility. The chip is organized into trenches shown schematically in Figure 1.4. Prior to running an assay, the bottoms of the trenches are functionalized with target specific capture probes. Following incubation with the sample, magnetic bead assay labels are allowed to sediment on the surface of the chip where they bind either strongly via specific interactions indicating the presence of analyte target, or weakly through non-specific Derjaguin, Landau, Verwey and Overbeek (DLVO) forces. Weakly bound bead labels are removed from the center of the trenches by passing current through the separation conductors embedded in the ridges along the sides of the trenches. Finally, the strongly bound magnetic beads remaining in the center are magnetized by micro-coils and detected by Hall sensors embedded in the substrate.



Figure 1.4: Surface of CMOS IC capable of autonomously performing a bio-assay

Chapter 2 explores the trade-offs of on-chip magnetic separation of magnetic beads and presents a design that is capable of discriminating specifically from non-specifically surface bound beads. Chapter 3 describes a fully integrated scheme for the detection of surface bound magnetic beads using CMOS. Chapter 4 demonstrates the design and performance of the first integrated CMOS-based assay platform that combine the functionalities of the previous two chapters. Chapter 5 will show initial work towards a low cost, easy to use membrane filtration based sample preparation system that leverages the functionality of the assay chip.

The sample preparation and the integrated assay platform can be combined in a fully integrated digital platform, shown in Figure 1.5., capable of performing multiplexed, quantitative bio-assays. Such a device has the potential to overcome the existing market barriers to de-centralization of medical care.



Figure 1.5: Stand-alone digital PON IVD tester

Chapter 2

2 On-chip magnetic bead separation

2.1 Introduction

ICs have been proposed as candidate platforms with which to perform magnetic bead labeled assays¹⁷. In such assays, specific bio-molecular complexes strongly tether magnetic beads to the surface of an IC that contains magnetic sensors. Before the detection of the strongly bound beads can occur, the interfering signal from weakly non-specifically bound ones must be eliminated. The problem of removing the weakly bound magnetic particles remains a key hurdle to implementing effective PON IVD platforms.

One possibility consists of using hydrodynamic forces to wash away weakly bound magnetic bead labels¹⁸. To do so, the IC must be integrated into a micro-fluidic cartridge, which limits the ease-of-use and adds significantly to the system complexity, resulting in higher overall system cost.

Another alternative is to use magnetic forces to remove the non-specifically bound beads from atop the magnetic sensors. Several groups have used external permanent magnets to generate the magnetic forces to remove the non-specifically bound beads from the surface of the IC^{17,19}. However, this off-chip magnetic separation requires additional handling steps that can complicate the assay protocol and impact ease-of-use.

Lee et al.²⁰ demonstrated a CMOS platform capable of electronically manipulating individual magnetic beads, but this functionality was never applied to bio-assay applications.

Osterfeld et al.²¹ separated magnetic particle labels thermodynamically. Small 50nm magnetic particles labels remained in aqueous suspension unless bound to the surface of the chip through a complementary bio-chemical interaction. Unfortunately, this method suffered from two major drawbacks; 1) the slow diffusion processes of the magnetic particles can lengthen the assay times for high sensitivity applications, and 2) the use of small particles greatly complicate on-chip detection. This limitation will be expanded upon in the following chapter.

In this chapter we present an assay cartridge consisting of a gold coated $0.18\mu m$ Complementary Metal Oxide Semiconductor (CMOS) IC capable of generating on-chip magnetic forces that are used to a) concentrate sedimenting $4.5\mu m$ super-paramagnetic beads over the detection area on the surface of the IC, and to b) remove non-specifically bound magnetic beads from the detection area²². The detection range and the sensitivity of a Human immunoglobulin G (IgG) immuno-assay using on-chip magnetic separation are compared to conventional ELISA. Additional control assays are performed to quantify the effects of magnetic bead labeling and the use of gold surface for passive protein adsorption.

2.2 System Overview

The surface of the CMOS IC used to demonstrate on-chip magnetic bead assay label separation is shown in Figure 2.1. Magnetic beads sedimenting to the gold coated surface of the IC are initially drawn by magnetic force towards the concentration conductor through which current flows. The separation conductors are placed along the upper ridges of trenches etched into the SiO₂, above the plane onto which the magnetic beads settle to eliminating the component of the magnetic force that pulls the beads into the surface of the IC and that can lead to inconsistent separation performance²³. After magnetic separation, only the strongly bound beads remain in the center of the trench. For verification of magnetic separation, the beads in the center of the trench are detected optically through a microscope and counted.



Figure 2.1: On-chip magnetic separation platform

2.3 System Design

2.3.1 Post Processing and Assembly

The CMOS compatible post processing steps for etching the trenches and for depositing the layer of gold are depicted in Figure 2.2; the 0.18µm 5 metal layer CMOS IC has Top Metal features to define the trenches. Single, diced ICs were first mounted

onto a 4" holding wafer using a drop of I-line photoresist applied manually with a thin brush. The photoresist was hard-baked for 1 hour at 120°C. Photoresist from that same pen was then applied to the areas of the IC that needed to be protected from the RIE (pads, exposed circuits etc.). The newly applied photoresist was again hard-baked for 1 hour at 120°C. The wafer was placed in the RIE chamber (AutoEtch Plasma Etch System, Lam Research) to remove the exposed SiO_2 . The plasma was fired for 20 seconds at a time, 12 times, with 90 second intervals in between to allow the sample to cool. The Top Metal features define the trenches while Metal 2 features were used as the etch-stop layer at the bottom of the. The wafer with the mounted ICs was then dipped in an aluminum etch for 2 minutes (80% H₃PO₄, 5% HNO₃, 5% CH₃COOH, 10% DI, Transene Company, Inc). The photoresist was stripped using PRS-3000 (J.T. Baker) heated to 90°C. This step removed the photoresist from the IC and also released the IC from the wafer. Individual ICs were then rinsed in ethanol followed by DI water and dried. The ICs were then placed upside down in a 4" machined aluminum wafer shadow mask that had slots for the ICs on one side and holes exposing the trenches at the center of the IC. This wafer was placed inside an evaporator (AUTO 306 Vacuum Chamber & EB3 Multi hearth Electron Beam Source, BOC Edwards) which was pumped down to 10⁻⁵ Torr. Then 10nm of Cr followed by 30nm of Au were deposited through the shadow mask. Figure 2.3 shows an SEM of the IC after 4.5µm M-450 Dynal magnetic beads were applied in solution and dried on the surface.

Four 200µm long trenches are etched into the surface of the IC, of 20µm, 24µm, 28µm and 32µm widths. The different widths were used to optimize the performance of the magnetic separation and minimize the power consumption. The 28µm wide trench proved most effective since the wider trench required more power to separate magnetic beads, while the narrower trenches were less effective at magnetic separation; non-specifically bound beads occasionally accumulated 2 wide along the sides of the narrower trenches and infringed onto the detection area in the center.

Figure 2.4 shows a micrograph of the CMOS IC with which the on-chip magnetic concentration and magnetic separation are performed. As in Aytur et al.¹⁹, the ICs are flip-chip bonded to the bottom of a Printed Circuit Board (PCB) after post processing. The top of the PCB houses a 150µl well with an aperture at the bottom to allow fluids to reach the trenches. Duralco 4525 epoxy is flowed via capillary force between the IC and the PCB to isolate the electrical flip-chip connections from the conductive fluid in the wells. The PCBs were manufactured by Hughes Electronics and the assembly was performed at Aspen Technologies (Figure 2.4). After several iterations, a 94% manufacturing yield was achieved (30 functional parts out of 32), including post-processing and assembly.

In this work, Dynal M-450, 4.5μ m in diameter, super-paramagnetic beads are used¹⁹. These beads, characterized in Fonnum et al.¹⁵, are spherical polymer matrices containing 20% maghemite by weight in the form of 8nm particles.



Figure 2.2: Cross sectional view of post processing steps of magnetic separation IC a) Standard 5 metal layer 0.18 μ m CMOS IC. b) Reactive Ion Etch (RIE) c) Aluminum wet etch d) Photoresist removal and deposition of Cr followed by Au through a shadow mask.



Figure 2.3: SEM of post-processed IC



Figure 2.4: CMOS IC and assay cartridge. a) Micrograph of 0.18µm CMOS IC with magnetic bead concentration and separation capabilities. b) Picture of the assembled device

2.3.2 On-Chip Magnetic Concentration

Figure 2.5a shows a cross section of a trench with magnetic bead settling out of solution due to gravitational forces. Current passing through a concentration conductor embedded under the center of the trench generates a magnetic force that draws the bead towards the center. The detection area where beads are optically counted is defined as the 10µm wide strip along the center of the trench. The concentration of magnetic beads to the detection area confers several advantages: 1) control over the location where the magnetic beads settle on the IC allows for the application of more precise magnetic separation forces, to better discriminate between the weakly bound and strongly bound magnetic beads, 2) by increasing the surface concentration of beads atop the magnetic sensors, fewer beads can be used, and 3) on-chip magnetic concentration can be used to pull the magnetic beads directly over magnetic bead sensing elements.

The acceleration of the beads sedimenting to the surface of the IC, $a_{sediment}$, as a function of time *t* is given by:

$$a_{sediment}(t) = \frac{F_{gravity} - F_{drag}}{m_b},$$
(4)

where the effective mass of the bead in water, $m_{bead} = 29$ pg, and the gravitational force on a Dynal M-450 bead, $F_{gravity}=0.28$ pN. The viscous drag force, F_{drag} , is expressed as a function of the sedimentation speed, $v_{sediment}(t)$, by

$$F_{drag} = 6 \cdot \pi \cdot \eta \cdot r_{bead} \cdot v_{sediment}(t), \tag{5}$$

where the viscosity of water $\eta = 0.89$ g/m*s and the radius of the bead $r_{bead} = 2.25 \mu m$. By combining Eqs. (4) and (5), we get the following expression for $v_{sediment}(t)$:

$$v_{se \dim ent}(t) = \frac{F_{gravity}}{6 \cdot \pi \cdot \eta \cdot r_{bead}} \left(1 - e^{\frac{-6 \cdot \pi \cdot \eta \cdot r_{-}}{m_{-}}t} \right)$$
(6)

The time constant of acceleration time constant $\tau = m_{bead}/6 \cdot \pi \cdot \eta \cdot r_{bead}$ is approximately 0.7µs, so we can approximate Eq. (6) by:

$$v_{se\,dim\,ent}(t) \approx \frac{F_{gravity}}{6 \cdot \pi \cdot \eta \cdot r_{bead}} \tag{7}$$

According to Eq. (7), the 4.5μ m beads settle out of solution to the surface of the IC at a rate of approximately 0.4mm/min. It is important to note that the steady state sedimentation rate is proportional to the square of the radius of the bead. Therefore, the size of the magnetic beads is an important factor for application where long assay times are unacceptable.

The magnetic beads approaching the surface of the IC are pulled towards the concentration conductor embedded on-chip. The magnetic force, F_{mag} , on a superparamagnetic bead with susceptibility χ_{bead} and radius r_{bead} from current I_{sep} passing through a straight conductor at a distance x_{bead} is derived in Shevkoplyas et al.²⁴;

$$F_{mag} = \frac{\mu_o \cdot \chi_{bead} \cdot r_{bead}^3 \cdot I_{sep}^2}{3\pi \cdot x_{bead}^3} \,. \tag{8}$$

The concentration conductors are implemented in Metal 1 and placed 0.75µm underneath the detection area in the center of the trenches. During the magnetic bead concentration process, 2mA flowing through the 2µm wide concentration conductors pulls the beads towards the detection area with a force of approximately 0.2pN from a distance of $x_{bead} = 4\mu$ m (Figure 2.5a). The magnetic force decays with the cube of the distance from the bead to the concentration wire, so long range on-chip magnetic concentration is not possible, i.e. for $x_{bead} > 100 \mu$ m. Rather, gravity is used to bring beads to the surface of the IC and on-chip magnetic concentration is used to guide those beads to the detection area.

2.3.3 **On-Chip Magnetic Separation**

After concentration, the non-specifically or weakly bound beads in the detection area are pulled aside by a magnetic force generated by current passing through a separation conductor embedded along the upper ridges of the trenches, as shown in Figure 2.5b. Note that the negative z-component of the magnetic force that would normally pull the magnetic beads into the surface of the IC is eliminated. For the 28 μ m wide trench, the distance between the center of the trench and the center of the separation conductor is 18 μ m.

To perform an immuno-assay, the magnetic forces must be sufficiently strong to pull the non-specifically bound beads to the side of the trench (i.e. > 0.1-10pN)¹⁸, away from the detection area, but not overly strong so as to remove the immunologically bound ones (i.e. < 60pN)²⁵. According to Eq. (8), 50mA of current flowing through the separation conductors pulls a magnetic bead resting in the center of the trench with 1.1pN of force. However, since the beads are pivoting on the surface of the IC as they roll, the magnetic separation force on the last molecular tether is amplified by the mechanical leverage effect resulting from the difference in lengths of the moment arms of the molecular tether and the separation force¹⁸ (Figure 2.6). The expression of F_{tether} the force on the last molecular tether of length *L* is given by:

$$F_{tether} = F_{mag} \sqrt{\frac{r_{bead}}{2L}} , \qquad (9)$$

In this case, the longest possible non-specific tether consists of 3 cross-reactive antibodies, which total 25.5nm in length²⁶. The 1.1pN lateral magnetic force translates

into a tensile 7.5pN force on the last tether, sufficient to remove non-specifically bound beads.

Eq. (4) shows that the force decays with the cube of the distance, hence to ensure that the magnetic beads are removed uniformly from the detection area, current is alternated between the separation conductors on either side of the trench. These conductors are addressed by decoding circuitry embedded in the chip to permit digital modulation of the currents.



Figure 2.5: Cross section of trench etched in SiO₂



Figure 2.6: Mechanical leverage effect

2.3.4 Joule Heating

To avoid denaturing the proteins at the surface of the IC that occurs when temperatures reach 40° C, the joule heating from passing 50mA through the 2µm wide, 0.5µm high and 200µm long conductors must be minimized. The heat generated is dissipated in several ways, the two most important being transfer through the IC and storage in the thermal capacitance of the fluid in the well. Figure 2.7 shows a cross section of the magnetic separation conductors with the equivalent thermal circuit of the IC. The equation for the heat transfer is given below:

$$\Delta T = \left(T_{final} - T_{ambient}\right) \left(1 - e^{-t/\tau}\right)$$
(10)

where the steady-state final temperature $T_{final}=P_{in}(R_{th2,SiO2}+R_{th,Si})$ and the thermal time constant $\tau = (R_{th2,SiO2} + R_{th2,SiO2}+R_{th,Si})C_{th,fluid}$ when only accounting for these two major sources of heat transfer. Calculations show that the implementation has a thermal time constant $\tau = 193$ s. A magnetic separation power $P_{in} = 20$ mW for 30 seconds will increase the temperature of the fluid in the well by 2.2°C above ambient temperature $T_{ambient}$, which will not affect the characteristics of the proteins on the surface.



Figure 2.7: Lumped equivalent thermal circuit

2.3.5 Choice of Magnetic Beads

Compared to the smaller beads used in most on-chip magnetic bead labeled $assays^{17}$, larger forces can be exerted on the 4.5 µm-wide beads. This is due to their higher volume magnetization and to the higher mechanical leverage on the tether bond. Power consumption and by extension heat dissipation can be reduced through shorter separation times, lower separation currents or a combination of both. Therefore, for this proof-of-concept, the 4.5µm-wide Dynal M-450 magnetic beads are chosen. However, the use of these larger beads does have disadvantages, notably;

- 1- The larger contact area between the beads to the surface of the IC leads to more non-specific interactions and therefore to higher non-specific binding forces. This is mitigated through surface bio-chemical passivation techniques and by rolling the beads horizontally across the surface of the IC, away from the detection area, as opposed to removing them vertically using a permanent magnet.
- 2- The resolution and the dynamic range of the bead labeled assay are limited by the total number of beads that can bind to a given detection area. Larger beads imply lower resolution and dynamic range for a fixed detection area and ideal detection.

2.3.6 Chip and Bead Surface Bio-Functionalization

The bio-chemical sensitivity of the on-chip protocol is determined by assaying known concentration of purified Human IgG. Figure 2.8 depicts the nature of the immunological sandwich capture complex immobilizing the beads to the detection area. The steps to coat the surface of the chips are enumerated below;

- 1. The gold surface is first coated with surface antibodies, polyclonal goat IgG specific to the F_{ab} region of Human IgG (Sigma Aldrich).
- 2. The surface is then bio-chemically passivated with non-fat dried milk (NFDM).
- 3. Varying dilutions of the Human IgG (Sigma Aldrich) antigen are introduced onto the chip.
- 4. The primary antibody, a biotinylated monoclonal goat IgG specific to F_c region of Human IgG (Sigma Aldrich) is incubated.

Meanwhile, the surface of the streptavidin coated 4.5µm Dynal bead labels are passivated by a solution of PBS with NFDM and Bovine Serum Albumin (BSA, Sigma Aldrich).

Once both the bead and chip surface are bio-functionalized, the assay protocol described in Section 2.2 can begin.

2.3.7 Control Assays

The on-chip assay conjugation immuno-chemistry presented in this work differs from that of conventional ELISAs in two ways; 1) ELISAs use enzymatic labels that catalyze a phosphorescent substrate while the on-chip assays use magnetic bead labels, and 2) ELISAs are performed on polystyrene surfaces, while the on-chip assays are performed on the gold coated IC. Several sets of control assays are performed to quantify the effects of these variables;

- 1. Conventional ELISAs on polystyrene 96 well plates are used as the baseline for evaluating the performance of all of the assays.
- 2. ELISAs on gold coated silicon slides are used to determine the performance of assays run on gold surfaces versus assays run in polystyrene wells.
- 3. A standard magnetic bead labeled assay is used to determine the performance of magnetic bead labeling versus enzymatic labeling. Nonspecifically bound beads are removed with a permanent magnet. In this control, Dynal M-280 2.8µm-wide magnetic beads were used rather than Dynal M-450 4.5µm-wide beads, since the latter aggregate when magnetically separated using a permanent magnet due to the strong fringe magnetic fields.





2.4 Assay Protocols

2.4.1 Polystyrene Control ELISA

All proteins were diluted in PBS unless otherwise specified. Conventional ELISAs on a polystyrene surface were performed in a 96-well polystyrene plate. Wells were incubated with 100µl of anti-Human Fab-specific IgG manufactured in goat (Sigma Aldrich) for 3 hours at room temperature, at a concentration of 5.3µg/ml. The solution was then discarded, and the wells blocked with 120µl of 0.3% NFDM for 16 hours at 4° C. Wells were then washed 3X in 200 µl of 0.50 PBS-Tween, and cleaned wells were incubated with 100µl of purified Human IgG (Sigma Aldrich) for 1 hour at room temperature. Ten-fold dilutions of purified Human IgG were tested ranging from 1mg/ml down to 100pg/ml along with a no-IgG control. After incubation with Human IgG wells were washed 4X in PBS-Tween, then incubated with 100µl of 500ng/ml biotinylated anti-Human Fc-specific IgG manufactured in goat (Sigma Aldrich) for 1 hour at room temperature. This was followed by another 4X wash in PBS-Tween. Wells were then incubated with 100ul of 2.8 µg/ml streptavidin-linked alkaline phosphatase (Pierce Biotech) diluted in PBS-Tween for 1 hour. After washing the wells 5X with PBS-Tween, 100µl of para-nitrophenyl phosphate (pNPP, Pierce Biotech) were added to each well in quick succession, and the catalytic reaction was allowed to progress for 1 hour. At this point the reaction was halted with 100µl of 3M NaOH, and the plates were read on a BioTek EL808 ELISA reader at a wavelength of 405 nm.

2.4.2 Gold Surface Based Control Assay Preparation

Experiments performed on gold surfaces were performed by cutting gold-coated silicon wafers into slides. The resulting slides were washed gently with 100% ethanol and rinsed with DI water, then blown dry. Cleaned slides were affixed onto a 16-well ProPlate Multi-Array Slide Module (Grace Bio-Labs). Wells were filled with 130µl of anti-Human Fab-specific IgG manufactured in goat (Sigma Aldrich) for 3 hours at RT at a concentration of 5.3μ g/ml, then blocked with 150µl of 0.3% NFDM for 16 hours at 4°C. The wells were then washed 3X in PBS-Tween and incubated with 130µl of purified Human IgG (Sigma Aldrich). Eight separate wells were used for each serial 10-fold dilution of Human IgG, ranging from 1µg/ml to 100pg/ml with a dilution factor of 1/10 for 5 aliquots total. After washing 4X in PBS-Tween, the wells were incubated with 100µl of 500 ng/ml biotinylated anti-Human Fc-specific IgG manufactured in goat (Sigma Aldrich) for 1 hour at RT. A 4X PBS-Tween wash prepared the samples for either a gold-surface ELISA or a bead-count assay.

2.4.3 Gold Surface Based ELISA Labeling and Detection

Samples were incubated with 130µl of 2.8μ g/ml streptavidin-linked alkaline phosphatase (Piece Biotech) diluted in PBS-Tween for 1 hour. After washing the wells 5X in PBS-Tween, 130µl of pNPP were added to each well in quick succession and the catalytic reaction was allowed to progress for 1 hour. 100µl of the fluid was then pipetted quickly to a 96-well polystyrene plate and the reaction was halted with 100µl of 3M of NaOH so that it could be read in a BioTek EL808 ELISA reader at a wavelength of 405nm.

2.4.4 Magnetic Bead Based Control Assay Labeling and Detection

Streptavidin-coated paramagnetic 2.8μ m (M-280 Dynabeads, Invitrogen) were blocked at a 1:1:1:7 proportion of beads:0.5% NFDM:0.5% BSA:PBS for 3 hours on slow rotation to keep the beads in continual suspension. The beads in blocking solution were then diluted by a factor of 2/5, so that the final concentration of beads was 1/25 of the original stock solution. 100 µl of this solution was pipetted into the wells in sets of 8, and the beads were allowed to settle for 10 minutes before they were washed with a row of 6/16-inch neodymium rare-earth block magnets (K&J Magnetics) attached side-toside, with magnetic poles directed up and down. The row of magnets was gently slid back and forth over the tops of the wells 3 times, then allowed to rest for 2 minutes to remove the nonspecifically-bound beads. The magnets were then moved aside to trap the freshly washed to the side of the ProPlate well, and 3 non-overlapping pictures corresponding to 1mm² were taken in the centre of each well at 200X magnification using a CCD camera (Micromanipulator Co. Inc., Moticam Inc.). The beads present in the 3 pictures from each well were counted and added together automatically using a MatLab application. It is important to note that the beads used in this control assay were 2.8µm Dynal M-280 rather than the 4.5µm Dynal M-450 beads used for the on-chip assay. The 4.5µm beads produce strong fringe force fields when exposed to the magnetic washing field from a permanent magnet, resulting in aggregation of large numbers of beads and invalidation of the assay. Nonetheless, the 2.8µm beads give a reasonable indication of the performance of bead based assay labeling.

2.4.5 On–Chip Purified Human IgG Assay

Streptavidin-coated 4.5µm (M-450 Dynabeads Invtrogen) were blocked in a 1:1:8 solution of beads:5% NFDM:PBS for 3 hours on slow rotation. The PCB wells were incubated with 100µl of anti-Human Fab-specific IgG manufactured in goat (Sigma Aldrich) for 3 hours at room temperature, at a concentration of 5.3µg/ml. The solution was then discarded, and the wells were blocked with 120µl of 0.3% NFDM for 16 hours at 4°C and then washed 3X with 200µl of 0.05% PBS-Tween. Experiments with different aliquots of Human IgG and were performed serially on a single device, starting with the negative control, to minimize the total number of devices needed. For the negative control aliquot, 100µl of 0ng/ml of Human IgG was introduced into the well and incubated for 1 hour at room temperature. After incubation, wells were washed 4X in PBS-Tween, then incubated with 100µl of 500 ng/ml biotinylated anti-Human Fc-specific IgG manufactured in goat (Sigma Aldrich) for 1 hour at room temperature. This was followed by another 4X washing step in PBS-Tween. 40µl of a 1/50 dilution of blocked streptavidin 4.5µm beads were added to the wells and let settle for 2.5 minutes with 2mA of current flowing through the concentration line. Afterwards, the magnetic washing conductors on alternating sides of each trench were pulsed with 50mA of current at 0.3Hz for 30 seconds. A picture of the 28 µm trench was taken and the number of beads left in the detection area was visually counted.

After the negative control, the well was washed 3X with PBS-T to remove all the beads from the surface of the IC. The next dilution of Human IgG was introduced into the well in increasing order, from 1ng/ml to 100ng/ml in 10-fold increments, and the assay protocol was resumed from the aliquot incubation step. This procedure was repeated three times until all the aliquots were assayed. Saturation of the antibodies at the surface of the IC was not observed since the number of beads remaining bound to the center of the trench increased with Human IgG titer.

2.5 Results

2.5.1 Control Assays

Standard curves for serial 10-fold dilutions of purified Human IgG from 1μ g/ml to 100pg/ml were produced using conventional ELISA on polystyrene, ELISA on gold and the off-chip magnetic bead labeled assay. The experiment at each dilution was repeated 8 times and the error bars correspond to +/- 1SD. Figure 2.9 shows the comparison of the biochemical sensitivity and dynamic range of the three protocols. The

conventional ELISA had a sensitivity limit of 10ng/ml, with an upper detection range at 100ng/ml. ELISA on gold performed better; concentrations down to 1ng/ml were detected reliably with an upper detection range of 100ng/ml. A possible explanation for the improved performance may be that gold surfaces have better passive protein adsorption properties than polystyrene surfaces. The Dynal M-280 bead labeled assays performed similarly to ELISA on gold, with a sensitivity limit of 1ng/ml and an upper detection maximal at 100ng/ml.

Of importance, the ratio of the 100ng/ml signal to its respective negative control for the magnetic bead based assays was over twice higher than the ELISAs, and the linear dynamic range of the bead assays extended to lower concentrations, suggesting that superior quantitative resolution can be achieved through magnetic bead labeling.

Due to the shorter antigen incubation time (1 hour versus 16 hours), the sensitivities of the control assays presented in this work are below those presented in Aytur et al., 2006. These results are nonetheless consistent with rapid, compact, commercially available ELISA protocols²⁷.

2.5.2 Magnetic Bead Sedimentation and Concentration

The measured settling rates for the M-450 magnetic beads and the M-280 beads settled were 0.32mm/min and 0.07mm/min respectively. Figure 2.10 shows the time lapse micro-graphs of the 28µm by 200µm trench, taken over 2.5 minutes, as M-450 beads sedimented to its surface. The concentration conductors had 2mA of current flowing through them to pull the beads to the 10µm wide detection area delineated by the shaded rectangle. In 4 experiments without magnetic concentration, only 36% of the beads landing in the trench landed in the detection area, close to the *a priori* ratio of the detection area to the area of the entire trench. On the other hand, in 4 experiments where 2mA of current flowed through the magnetic concentration conductor, the proportion of beads landing in the detection area increased to 55%. Table 3.1 presents the concentration statistics. Higher magnetic concentration forces resulted in more efficient magnetic bead concentration but the use of currents in excess of 2mA led to stronger non-specific interaction between the beads and the surface of the trenches, leading to a loss in assay sensitivity. This effect may be mitigated through better surface functionalization techniques discussed in Section 5.6.3.

	Total Number of Beads that		Proportion of Beads that Settled	
	Settled in Detection Area		in the Detection Area	
	Number	Std. Dev.	%	Std. Dev.
Without Magnetic	21.75	1.5	36.4%	0.06%
Concentration				
With Magnetic	28.75	4.7	55.3%	0.08%
Concentration				

 Table 2.1: Magnetic concentration results for the 28um wide trench



Figure 2.9: Control assays results

2.5.3 On-Chip Magnetic Bead Separation

The on-chip magnetic separation was started immediately after the 2.5 minutes needed for the magnetic beads to settle. The few beads that had not settled prior to the magnetic separation were simply pulled towards the magnetic separation conductors, away from the detection area in the center of the trench. Figure 2.11 shows the micrographs of a trench before (*left*) and after magnetic separation (*right*) for Ong/ml, 1ng/ml and 10ng/ml concentrations of purified Human IgG antigen.

A separation current of $I_{sep} = 50$ mA for a duration of $t_{sep} = 30$ seconds was needed to remove all the non-specifically bound beads from the detection area for the negative control. To ensure that the beads were removed symmetrically from the center of the trench, I_{sep} was digitally alternated between the left and right separation conductors at a frequency $f_{sep} = 0.3$ Hz. Force modulation frequencies over 1Hz were not effective for removing beads from the center of the trench since inertial forces high pass filter the motion of the beads. Frequencies below 0.1Hz were also less effective since there were fewer number of transitions in the direction of the magnetic force that "loosen" the nonspecifically bound beads through high loading forces. For $I_{sep} = 50$ mA the separation time was limited to 30 seconds after which time no bead displacement was observed.


Figure 2.10: On-Chip magnetic bead concentration



Figure 2.11: On-chip magnetic bead separation

The 28μ m wide trench was optimal for assays; wider trenches required higher separation currents resulting in higher power dissipation, while in narrower trenches, aggregation of the 4.5 μ m beads along the sides of the trenches overflowed into the detection area.

2.5.4 On-Chip Human IgG Assay Results

The standard curves for 10-fold dilutions of purified Human IgG from 100ng/ml to 1ng/ml and one negative control were produced using on-chip magnetic separation. Figure 2.12 presents the results from two independent on-chip assay experiments, performed on separate days, while Figure 2.13 shows the comparison between the conventional ELISA on polystyrene and the average of the two on-chip assays. Purified Human IgG was detectable down to concentrations of 1ng/ml, or 7pM, with a correlation factor of 0.995 to the ELISA. The on-chip assay sensitivity is higher than conventional ELISAs in spite of a much smaller assay surface area; one 28µm by 200µm trench for the on-chip protocol as opposed to 97mm² for the ELISA. The strong correlation to ELISA suggests that on-chip magnetic bead separation does indeed have the ability to separate specifically bound magnetic bead labels from non-specifically bound ones. For confirmation and to eliminate the variability in the total number of beads that land on the surface of the trench, Figure 2.14 presents the bead binding effectiveness, i.e. the percentage of beads remaining in the detection area after magnetic separation for different IgG concentrations. It is interesting to note that while the standard curves individually correlate very well with ELISA, they are offset from each other, suggesting that there is high variability assay run to run. The causes of these inconsistency and possible mitigating factors will be further explored in Section 4.5.3.

This magnetic bead separation scheme cannot discriminate between beads bound to the surface of the IC through one or more specific immunological interactions, since the molecular tethers are disrupted one at a time as the beads roll of the center of the trench. This limitation results in a compressed detectable dynamic range but does not impact detection sensitivity. To acquire bond multiplicity information, the beads must be pulled vertically using a permanent magnet or Atomic Force Microscope tip¹⁷.

Nonetheless, the high sensitivity, the low cost and the ease-of-use of on-chip magnetic bead separation technology makes it a viable candidate for PON IVD use.



Figure 2.12: On-chip assay results



Comparison to Standard ELISA on Polystyrene

Figure 2.13: Comparison of the average of the 2 on-chip assays with ELISA on polystyrene



Relative Separation Performance

Figure 2.14: Relative on-chip magnetic bead separation performance

2.6 Conclusion

We have successfully demonstrated on-chip magnetic separation of 4.5 um superparamagnetic beads weakly bound to the surface of a CMOS IC for integrated molecular analysis. Magnetic beads sedimenting via gravity to the surface of the IC were first concentrated to the 10µm wide detection area in the center of a 28µm wide by 200µm long trench etched in the SiO₂. The proportion of beads landing in the detection area rose from 36% without magnetic concentration to 55% with magnetic concentration. After sedimentation, the non-specifically bound beads were removed from the detection area by a magnetic force generated by passing 50mA of current through conductors embedded along the ridges of the trench, 18µm away. To ensure proper removal of the nonspecifically bound beads from the detection area, the separation current was digitally alternated between the two ridges at a frequency of 0.3Hz for 30 seconds. Due to mechanical leveraging, a 1.1pN lateral magnetic force translated into tensile 7.5pN on the bio-chemical tether immobilizing the bead. This on-chip magnetic separation functionality was applied to an immuno-assay on purified Human IgG samples and concentrations down to 1ng/ml or 7pM were detectable, with a correlation factor of 0.995 to standard ELISA. On-chip magnetic separation was shown to be a versatile alternative to conventional fluidic washing of assay labels. While the detection of the magnetic bead labels was performed optically, the next chapter will demonstrate a fully integrated magnetic bead detection system that can readily be integrated with the on-chip magnetic separation functionality.

Chapter 3

3 On-Chip Detection of Magnetic Beads

3.1 Introduction

The pioneering work on IC-based magnetic bead detection systems used Giant Magneto-Resistive (GMR) technology in combination with off-chip electro-magnets¹². Later implementations obviated the need for external electro-magnets, but continued to employ magneto-resistive technologies^{28,29}.

Similarly to the early works using GMR, the first CMOS bead detection systems relied on one or more external electromagnets^{14,19}. More recently, Wang et al.¹¹ presented a fully integrated CMOS platform consisting of on-chip planar coils whose inductance changed in response to the number of magnetic beads on its surface. However, this scheme could not easily be combined with on-chip magnetic separation since the size of the large coils would make removing the beads from their centers difficult, and more importantly since the magnetic beads pulled aside would add significantly to the background signal.

We present a fully integrated system capable of detecting single super-paramagnetic beads using CMOS that can be readily combined with the magnetic separation presented in the previous chapter³⁰. The external electromagnets that polarize the beads are integrated on-chip with the Hall sensors necessary for detection.

3.2 System Overview

In the detection system shown in Figure 3.1, an individual $4.5 \,\mu\text{m}$ superparamagnetic bead, immobilized by a specific bio-chemical tether to the surface of the CMOS IC, is magnetized by an applied magnetic field generated by current passing through an on-chip micro-coil. The induced bead magnetization field signal is electrically transduced by a planar n-well Hall sensor sensitive to vertical magnetic fields (i.e. in the z-direction), and amplifier on-chip. The current through the micro-coil is digitally modulated above the sensor and back-end circuit 1/f noise corner. For maximum signal, the distance between the Hall sensor and bead is reduced using the post-CMOS etch steps detailed in Section 2.3.1.



Figure 3.1: a) 3-Dimensional representation of a 4.5 µm bead above a Hall sensor/micro-coil pair, b) Micrograph top view of two Hall sensor/micro-coil pairs

3.3 System Design

3.3.1 Bead Magnetization Calculations

The integrated micro-coil that is used to magnetize the bead consists of a single current loop of inner radius a = 2.1um and a line width w = 0.5um, implemented in the lowest CMOS metallization layer. The z-component of the coil's applied magnetic field, $B_{applied}(z)$, can be described by the off-axis field of a current loop³¹:

$$B_{applied}(z) = \frac{\mu_o I_{coil}}{2\pi \sqrt{(a+r)^2 + z^2}} \cdot \left[E(k) \frac{a^2 - r^2 - z^2}{(a+r)^2 + z^2 - 4ar} + K(k) \right]$$
(11)

where μ_o is the permeability of free space, I_{coil} is the current through the coil, r is the distance from the center of the coil to the point of observation, E(k) and K(k) are the complete elliptical integral functions of the 1st and 2nd kind and k is given by:

$$k = \frac{4ra}{(a+r)^2 + z^2}$$
(12)

According to Eqs. (11) and (12), 16mA of current through the micro-coil produces a magnetic field $B_{applied}(bead) = 791 \,\mu\text{T}$ at the center of the bead and a field $B_{applied}(Hall sensor) = 1.7$ mT at the Hall sensor.

The bead magnetization vector field \mathbf{B}_{bead} resulting from the applied magnetic field $B_{applied}(z)$ is modeled by a perfect dipole moment¹⁴:

$$\mathbf{B}_{\text{bead}} = \frac{1}{4\pi} \cdot \frac{3(\mathbf{r} \cdot \mathbf{m}_{\text{bead}})\mathbf{r} - (\mathbf{r} \cdot \mathbf{r})\mathbf{m}_{\text{bead}}}{r^5}$$
(13)

r is the vector from the point of observation to the center of the bead, as shown in Figure 3.2. The bead's volume magnetization is given by $\mathbf{m}_{bead} = \chi_{bead} V_{bead} B_{applied}(z) \mathbf{z}$. The z-component of the applied magnetic field can be extracted from Eq. (13) and expressed as a scalar:

$$B_{bead,z}(x, y, z) = \frac{\mu_0 \cdot m_{bead}}{4\pi (x^2 + y^2 + z^2)^{3/2}} \cdot \left[2 \frac{z^2}{x^2 + y^2 + z^2} - \sin^2 \left(a \cos \left(\frac{z}{\sqrt{x^2 + y^2 + z^2}} \right) \right) \right]$$
(14)

For $B_{applied}(z_{bead})=1.7$ mT, Eq. (14) estimates the z-component of the induced magnetization field to be $B_{bead,z}(Hall \ sensor)=54$ uT across the contacts of the Hall sensor.



Figure 3.2: Bead magnetization dipole

According to Eq. (14), the bead magnetization field decays with the cube of the distance r, so the dielectric layer above the Hall sensor/micro-coil pair is etched back using the same procedure described in the previous chapter. After post processing, the distance is from the sensor plane to the center of the bead is reduce to 5.3 μ m.

The intensity of the z-component of B_{bead} versus lateral position in the plane of the sensor is plotted in Figure 3.3 using Eq. (14). The highly localized nature of the bead magnetization field implies that to obtain maximal Signal-to-Noise Ratio (SNR) the magnetic field detector must be as small as possible.



Figure 3.3: z-Component of the bead magnetization field at the Hall sensor plane

3.3.2 Bead Detection

The bead magnetization field is transduced by a cross shaped Hall sensor, shown in Figure 3.4, implemented in a 1µm-deep n-well diffusion with a sheet resistance of $800\Omega/\Box$. Current flowing from V_{DD} to ground in the presence of a magnetic field, results in a Hall Effect voltage across contacts A and B. The equation for the Hall sensor voltage V_H as a function of the z-component of the magnetic field is given by:

$$V_H = G_H \mu_H V_{DD} \frac{W_{Hall}}{L_{Hall}} B_z$$
(15)

 W_{Hall} and L_{Hall} are the width and length of the Hall plate, and the Hall mobility $\mu_H = 700 \text{ cm}^2/\text{V}$ s. G_H is the geometric factor of the Hall sensor, and is dependent on the length,

the width and the size of the contacts³². G_H decreases with the ratio of the length to the size of the contacts and since the minimum contact size is limited by process constraints to 0.18 µm on a side, the Hall sensor cannot be made arbitrarily small. In this implementation, the Hall sensor is 5.8 µm long by 5 µm wide with a geometric factor $G_H \sim 0.8$ and a calculated Hall sensitivity $S_V = 2.9\%/T$. The measured Hall sensor sensitivity is 2.7%/T for an input voltage of 2V and bias current of 2.2mA. The output impedance between contacts A and B is approximately 800Ω, while the total output impedance is 1kΩ including NMOS addressing switches, which corresponds to a 4nV/√Hz output thermal noise.

3.3.3 Back-End Electronics

The main difficulties with detecting the bead magnetization field are listed here; 1) the small 54uT bead magnetization field corresponds to ~1uV at the output of the Hall sensor, 2) the presence of low frequency 1/f noise, 3) the Hall sensor offset voltage $\sigma_{os}=5mV$ (sample size = 16), and 4) the applied magnetic field is 31 times larger than the in-phase induced bead magnetization field.





The weak output signal from the Hall sensor is amplified by a three stage Backend Amplifier (BA) providing 60dB of gain, with $4nV/\sqrt{Hz}$ of input referred thermal noise, 50kHz 1/f noise corner. The first stage of the BA consists of a low noise pre-amp providing 23dB of gain and dissipating 1.6mW. After amplification, the output is driven off-chip, through an RC low pass filter with a single pole at 250kHz, and into the 1MS/s 16-bit Analog-to-Digital Converter (ADC) of a 6259 NI DAQ (National Instruments).

The current through the micro-coils is chopped digitally at 50kHz, above the sensor and circuit 1/f noise corner frequencies. Routing to the Hall sensor is limited to Metal 1, so current commutating techniques to mitigate low frequency noise and offset would incur a large area penalty³³. It is important to note that solutions that rely on an external magnet to generate the polarization field usually are restricted lower frequencies, necessitating a second chopper to attenuate noise from the electronics¹⁰.

To eliminate any signal path DC offset that imposes large dynamic range constraints on the BA, the output of the pre-amplifier is AC coupled to the following gain stages. The single pole high pass filter corner frequency is set to 1kHz.

Another large dynamic range constraint comes from the in-phase applied magnetic field. To attenuate this signal, we employ a differential architecture that subtracts the signal of a replica Hall sensor with no bead atop it from the signal of Hall sensor exposed to beads. The replica sensor cannot detect a bead because it is placed in a location on the IC away from the aperture in the PCB exposed to the solution containing beads. Any residual signal due to mismatch between the two sensors is eliminated by auto-zeroing the output of the BA using an external feedback loop that applies an offset current in the replica coil. The block diagram of the differential architecture is presented in Figure 3.5, while the circuit level implementation of the differential signal path is presented in Figure 3.6.

The detection algorithm runs as follows: before the beads are applied, the exposed coil sees a fixed 16mA, 50kHz square current wave, while an off-chip feedback loop controls the amplitude of the 50kHz square wave calibration current through the replica coil to zero the output of the BA. After zeroing, the calibrated replica coil current is recorded and the system is ready for detection. Once a bead is applied on top of the exposed sensor, the calibrated current is driven through the replica coil and the new differential amplitude at the 50kHz fundamental is recorded. All off-chip detection circuitry, like the ADC and the calibration feedback loop, can be readily integrated on-chip in future implementations.

This calibration algorithm could be performed directly before use, or on the factory floor, without impacting ease-of-use. In fact, stand-alone operation greatly reduces testing overhead, since the PON IVD device can calibrate itself during the lengthy surface bio-functionalization steps performed on the factory floor. Furthermore, calibration can be used as an internal self test to meet the stringent quality control requirements imposed on all medical devices. Alternatively, better matching techniques can obviate the need for calibration altogether.



Figure 3.5: Differential architecture consisting of an exposed sensor (1), a replica sensor (2), a three stage Backend Amplifier and an off-chip 16-Bit ADC

3.4 Results

3.4.1 Single Bead Detection

A sensor was calibrated and subsequently exposed to a magnetic bead in solution. 16mA milliamps of current through a micro-coil produced an applied field of 1.47mT, and induced a bead magnetization field of 41.5μ T, as measured by the Hall sensor. The replica sensors, shielded from magnetic beads by a thick layer of dielectric, attenuated the large undesirable polarization field by 25dB. To enable unambiguous detection, the Hall sensor mismatch dominated residual offset was attenuated an additional 50dB, below the signal from the bead, by the calibration sequence. The comparison between calculated and measured magnetic fields is presented below in Table 3.1.

Figure 3.7 presents the spectral measurements in dBV at the output of the BA before calibration (top). The clearly distinguishable 50kHz square wave tones resulted from the mismatch between the exposed and replica Hall sensors signal paths. After calibration (middle) these tones were attenuated 50dB, into the noise floor. After application of a bead onto the exposed sensor, the tones that reappeared were a result of the bead magnetization field (bottom). This system was capable of detecting individual magnetic beads with 45dB of Signal-to-Noise Ratio (SNR) for a 1Hz noise bandwidth (i.e. for an integration time $\tau = 1$ s). With total input referred Gaussian noise of approximately 150nT/ \sqrt{Hz} , the probability of falsely detection a magnetic bead directly over the sensor is infinitesimally small.



Figure 3.6: Circuit schematic of the differential sensing element signal path

	Calculated	Measured
$B_{applied}(Hall \ sensor)$	1.7mT	1.5mT
$B_{bead,z}(Hall \ sensor)$	54uT	42uT

Table 3.1: Calculated, and measured z-component of the applied and bead magnetization field



Figure 3.7: Output of the ADC measured with a 1Hz noise bandwidth, before calibration (top), after calibration (middle), and after application of a bead (bottom)

3.4.2 Bead Detection Range

To quantify the bead detection range, a 4.5 μ m bead was dried on the surface of the IC and its position was moved in 1 μ m increments across the Hall sensor using a micro-manipulator. Figure 3.8 compares the measured bead magnetization fields with the values calculated by varying the position of the bead in Eqs. (11) and (14). A 10 μ T detection threshold, represented by the dashed line, corresponds to an 8 μ m-wide detection range. This 8 μ m bead detection range is ideal for integration with the magnetic separation system presented in the previous chapter, and can be modulated if necessary.



Figure 3.8: Hall sensor detection range

3.5 Conclusion

We have presented a fully integrated magnetic bead detection system that relies on a micro-coil and a Hall sensor that respectively magnetizes and detects a 4.5 μ m magnetic bead at the surface of a CMOS IC. The micro-coil consist of a 4.2um wide, single turn current loop implemented in the lowest metallization layer, generating fields of 1.5mT with 16mA of current. The 5 μ m wide by 5.8 μ m long n-well Hall sensor, used to detect the bead, has a sensitivity of 2.7%/T for an input voltage of 2V, and a bias current of 2.2mA. Using a differential amplifier with output auto-zeroing capability, single magnetic beads were detected with 45dB of SNR for a 1Hz noise bandwidth. Moreover, individual magnetic beads were detected at distances of up to 5 μ m from the center of the Hall sensor. Since this on-chip detection architecture does not reply on any key external components, it is well suited for integration with the on-chip separation presented in the previous chapter. The first fully integrated CMOS assay platform that combines both the on-chip magnetic bead detection and the on-chip magnetic separation will be presented in the next chapter.

Chapter 4

4 Fully Integrated CMOS Assay Platform

4.1 Introduction

Previous attempts to integrate assay label separation and detection into a single IC capable of autonomously performing an assay have necessitated either off-chip components or external fluidic handling.

The combination of electromagnetic magnetic bead separation and detection was first introduced by Baselt et al. in an assay platform that required external magnets to generate the separation forces and to magnetize the beads for detection¹⁷. Aytur et al. applied this protocol to CMOS¹⁹, but like Baselt et al.'s implementation, the need for external magnets greatly impacted PON utility.

Lagae et al. was the first to combine on-chip manipulation and detection of superparamagnetic beads²⁹. However, this platform did not conclusively demonstrate the ability to separate specifically from non-specifically bound magnetic beads. Instead, they presented a GMR implementation that required fluidic washing steps to remove the unbound magnetic particles.

Osterfeld et al. demonstrated a similar assay platform, but where the separation of 50nm magnetic particle labels was performed thermodynamically²¹. However, this system required a) an external electromagnet to strongly magnetize the small particles, and b) the use of GMR technology to detect the weak volume magnetizations of the particles bound to the surface of the chip.

Wang et al. presented a CMOS platform capable of detecting of magnetic beads bound to its surface without off-chip electro-magnets¹¹. In the absence of an integrated magnetic bead separation system, a micro-fluidic system with all its associated overhead was required to remove unbound beads.

The system presented in this chapter combines the magnetic bead separation and detection technologies of Chapters 2 and 3 in the first fully integrated assay platform. The bio-chemical sensitivity of the on-chip protocol was characterized by assaying known concentrations of purified Human IgG, a relevant target for a variety of infectious disease applications. The bio-chemical selectivity was demonstrated via an anti-dengue assay on dengue positive Human sera samples. Finally, a discussion will detail how this design fundamentally meets the key criteria of an effective PON IVD platform, namely high bio-chemical selectivity, low cost, ease of use and fast operation.

4.2 System Integration

4.2.1 Integrated Circuit

The general functioning of the bio-sensor front-end is described in Section 1.4. Aytur et al. demonstrated that 100 discrete sensors capable of detecting individual magnetic bead labels are sufficient for quantifying analyte down to the 7pM range¹⁹. Figure 4.1 presents the block diagram of the fully integrated assay chip fabricated in 0.18 µm CMOS, containing an exposed sensor bank with 128 Hall sensor/micro-coil pairs and a replica sensor bank with 16 Hall sensor/micro-coil pairs. As described in Chapter 3, the Backend Amplifier amplifies the difference between the signals from one addressed exposed Hall sensor and its corresponding replica sensor. The Current Control block delivers the calibrated, digitally modulated currents to the micro-coils, and also drives the separation currents. Lastly, to minimize the number of padring connections, a Serial Bus Interface parallelizes incoming serial digital data and holds the state.

The exposed sensor area consists of 16 25µm-wide assay trenches with 8 Hall sensor/micro-coil pairs aligned under the center of each trench, for a total of 128 individually addressable sensing elements, each capable of detecting single Dynal 4.5µm diameter M-450 super-paramagnetic beads.



Figure 4.1: Block level description of integrated assay platform

4.2.2 Post Processing and Assembly

The individual IC are post processed and assembled onto a PCB using the same manufacturing steps presented in Chapter 2. The cross section of the IC containing the micro-coils and Hall sensors is shown in Figure 4.2 through each step of processing.



Figure 4.2: Cross sectional view of the post processing steps of the fully integrated assay platform. a) Standard 5 metal layer 0.18µm CMOS IC. b) Reactive Ion Etch (RIE) c) Aluminum wet etch d) Photoresist removal and deposition of Cr followed by Au through a shadow mask.

4.2.3 Integrated Assay Algorithm

After cartridge assembly, the mismatches between the 128 exposed sensors and their corresponding replicas are attenuated sequentially by the detection calibration algorithm presented in the previous chapter. Calibration can be performed directly before use, or weeks in advance, since no appreciable drift was noted after 21 days.

The surface chip and bead bio-functionalization steps are enumerated in section 2.3.6. After surface preparation, the assay protocol is commenced; initially, each separation conductor is sequentially pulsed with current for 0.4 seconds. This cycle is repeated 10 times to ensure that all weakly bound beads are removed. Narrower 25μ m trenches are used in this implementation to reduce the heat dissipation and time needed to perform magnetic separate on all 16 trenches. According to the detection range measured in the Chapter 3, non-specifically bound beads can accumulate 2 wide along the side of the 25μ m trench without being detected. A 50mA current results in a 2pN lateral force on a bead in the center of the trench, 15μ m from the separation conductor. The mechanical leveraging effect from the bead pivoting on the surface of the trench translates the lateral force into a 13.2pN tensile force on the immobilizing molecular bond.

After on-chip magnetic separation, each Hall sensor is polled sequentially for 0.2 seconds. The amplitude of the 50kHz square wave resulting from the bead magnetization field is recorded, and the bead count is incremented if this magnetic field exceeds the threshold amplitude of 10μ T.

Both magnetic separation conductors, on either side of the Hall sensor being polled, have 25mA of current flowing through them during detection, to prevent unbound magnetic beads from being pulled back over the Hall sensors by the detection current flowing through the micro-coils.

The timing diagram for the calibration, separation and detection algorithms are shown in Figure 4.3. To upload a new state into the SBI controller, the data pin must be driven high for one clock cycle, indicating a framing bit. The following 16 bits of Data flopped into the 16-bit serial to parallel shift register determine the Hall sensor address and values of the currents.



Figure 4.3: Functional timing diagram

Figure 4.4 presents micrographs of the 2.5 x 2.5mm², 0.18µm CMOS IC capable of autonomously performing an assay, with an inset of an assay trench consisting of 8 inline micro-coil/Hall sensor pairs. The system setup, presented in Figure 4.5, consists of the assay cartridge that is connected, via an interface board containing the 250kHz antialiasing low pass filter, to the external 6259 NI DAQ that houses the 16-bit 1MS/s ADC. The NI DAQ also provides the input digital and current control signals to the IC, and transmits data to a laptop for storage and presentation.



Figure 4.4: Micrograph of 6.25mm² 0.18µm CMOS Assay platform with inlay of a trench containing 8 inline Hall sensors



Figure 4.5: Photograph of test setup

The bio-chemical sensitivity of this platform was evaluated by assaying known concentrations of purified Human IgG, while the bio-chemical specificity, i.e. the ability to identify a specific IgG idiotype from a larger ensemble population, was demonstrated by assaying Human sera samples for the presence of anti-Dengue IgG.

4.3 Assay Protocols

4.3.1 On-Chip Purified Human IgG Assays

Streptavidin-coated 4.5µm (M-450 Dynabeads Invtrogen) were blocked at a 1:1:1:7 proportion of beads:0.5% NFDM:0.5% BSA:PBS for 3 hours on slow rotation. The exposed sensors at the center of the PCB wells were dotted with 1µl of anti-Human Fab-specific IgG manufactured in goat (Sigma Aldrich) at a concentration of 5.3mg/ml and incubated overnights at 4°C. The top of the wells were hermetically sealed to prevent evaporation. The well were then washed 3X with 200µl of 0.05% PBS-Tween, blocked with 120µl of 0.3% NFDM for 16 hours at 4°C, and washed again 3X with 200µl of 0.05% PBS-Tween. 100µl of the Human IgG dilution was introduced into the well and incubated for 3 hours at room temperature. After incubation, wells were washed 4X in PBS-Tween, then incubated with 100µl of 100ug/ml biotinylated anti-Human Fc-specific IgG manufactured in goat (Sigma Aldrich) for 1 hour at room temperature. This was followed by another 4X washing step in PBS-Tween. 25µl of a 1/25 dilution of blocked streptavidin 4.5µm beads were added to the wells and let settle for 2.5 minutes. After magnetic separation, the beads remaining in the center of the trenches were detected electronically and optically using a microscope at 200x magnification.

4.3.2 Dengue Control ELISAs

The 96 well plate was coated with 50µl per well of 1µg/ml Dengue 1 purified E (Envelope) protein diluted in Carbonate Coating Buffer at a pH of 9.6 and refrigerate at 4 °C overnight. The next day, the wells were wash once with PBS-Tween, blocked with 200µl per well of 2.5% NFDM and 2.5% NGS diluted in PBS-Tween and incubated at room temperature for 3 hours. The wells were again washed once with 1x PBS-Tween before 50µl of sample diluted in PBS-Tween was added. The samples were 1:100 dilutions of serum from a dengue positive and dengue negative individuals, respectively. The sample was incubated in the well overnight at 4 °C. The next day, the wells were washed four times with PBS-Tween with a 5 minute wait between the 2^{nd} and 3^{rd} wash and the 3rd and 4th wash. 100µl were added in each well of 5.3µg/ml biotinylated Goat anti Human IgG and let incubate at room temperature for 1 hour. The wells were again washed four times with PBS-Tween with a 5 minute wait between the 2nd and 3rd wash and the 3rd and 4th wash. 100µl of 10µg/ml streptavidin were added in each well and incubated incubate at room temperature for 1 hour. The wells were washed four times with PBS-Tween with a 5 minute wait between the 2^{nd} and 3^{rd} wash and the 3^{rd} and 4^{th} wash. Lastly, 100µL of substrate pNPP were added in each well and developed for 30 minutes. The reaction was stopped with 50μ l per well of 3M NaOH and the results were read out on the spectrophotometer.

4.3.3 On-Chip Dengue Assays

Streptavidin-coated 4.5µm (M-450 Dynabeads Invtrogen) were blocked at a 1:1:1:7 proportion of beads:0.5% NFDM:0.5% BSA:PBS for 3 hours on slow rotation. Each assay cartridge was coated with 50µl per well of 1µg/ml Dengue 1 purified E (Envelope) protein diluted in Carbonate Coating Buffer at a pH of 9.6 and refrigerate at 4 ° C overnight. The next day, the wells were wash once with PBS-Tween, blocked with 200ul per well of 2.5% NFDM and 2.5% NGS diluted in PBS-Tween and incubated at room temperature for 3 hours. The wells were again washed once with 1x PBS-Tween before 50ul of sample diluted in PBS-Tween was added. The samples were 1:100 dilutions of serum from a dengue positive and dengue negative individuals, respectively. The sample was incubated in the well overnight at 4 °C. The next day, the wells were washed four times with PBS-Tween with a 5 minute wait between the 2nd and 3rd wash and the 3rd and 4th wash. 100µl were added in each well of 5.3µg/ml biotinylated Goat anti Human IgG and let incubate at room temperature for 1 hour. The wells were again washed four times with PBS-Tween with a 5 minute wait between the 2nd and 3rd wash and the 3rd and 4th wash. 100µL of 10µg/ml streptavidin were added in each well and incubated incubate at room temperature for 1 hour. The wells were washed four times with PBS-Tween with a 5 minute wait between the 2^{nd} and 3^{rd} wash and the 3^{rd} and 4^{th} wash. 25µl of a 1/25 dilution of blocked streptavidin 4.5um beads were added to the wells and let settle for 1.5 minutes. After magnetic separation, the beads remaining bound to the center of the trench were detected on-chip.

4.4 Results

4.4.1 On-Chip Assay Algorithm

Figure 4.6 presents the on-chip assay algorithm where magnetic beads first sedimented to the surface of the IC over 1.5 minutes (top). Afterwards, 50mA of current was passed sequentially through the magnetic separation conductors, one at a time (middle). Lastly, the beads remaining in the center of the trench were detected (bottom).

4.4.2 Magnetic Bead Detection

Figure 4.7 compares the performance of the on-chip magnetic bead detection versus optical detection of for a standard curve of serial 10X dilutions of Human IgG. In total, the detection accuracy exceeded 95%.



Figure 4.6: On-chip assay procedure



Human IgG Concentration

Figure 4.7: Comparison between optical and on-chip magnetic bead detection

4.4.3 On-Chip Purified Human IgG Assay Results

Serial 10-fold dilutions of purified Human IgG were assayed on-chip and the standard curves are presented in Figure 4.8. A separate chip was used for each data point, and all the points of the same standard curve were performed concurrently, but the three standard curves were performed on separate days.

The large variation between standard curves performed on separate days can be a result of several factors; a) the use of small number of sensors, b) variations in the onchip assay protocol, and c) variations in the run-to-run bead binding characteristics.

The use of only 128 sensors undoubtedly leads to statistical errors. However, the strong correlation between the standard curves and the rising IgG titer suggests that this is not the dominant effect.

The on-chip protocol consists of magnetic bead sedimentation, separation, and detection steps. To remove the variations resulting from the total number of beads sedimenting in the 8μ m wide detection area, and the variations resulting from inaccurate detection, Figure 4.9 measures the binding efficiency of the beads, i.e. the number of beads detected optically before and after magnetic separation. While the overall variability in Figure 4.9 between standard curves is reduced, it is clear that variability in the magnetic bead sedimentation and detection accuracy are only secondary factors. One must also assume that the variability resulting from the magnetic separation step is small due to the precision with which the separation forces are applied.

The standard curves correlated well individually with the rising IgG titer, but are offset from each other, suggesting that difficulty controlling the surface binding characteristics from run-to-run was the source of error. This same observation was made in the magnetic separation experiments of Chapter 2 (Figure 2.14), and this phenomenon was also noted by Liu et al.²³

In spite of this variability, a statistical relevance *t*-test (two-sample unequal variance with 2 distribution tails) comparing the negative control and the 100pg/ml data sets resulted in a value of 0.07, suggesting that this on-chip assay protocol was capable of detecting 100pg/ml concentration of Human IgG, or 700fM. This is superior to the 35pM sensitivity of conventional ICTs².

Furthermore, the on-chip standard curves are all monotonically increasing past the 1ng/ml concentration, suggesting that quantitation is possible between 1ng/ml and 100ng/ml with comprehensive on-chip controls, such as multiplexed high and low curve calibration points. Figure 4.10 compares the average of the on-chip assay standard curves to the conventional ELISA on polystyrene presented in Figure 2.9. The correlation coefficient between the two is R^2 =0.94.

It is important to note that the proportions of beads remaining in the center of the trench, shown in Figure 4.9 do not completely correspond with those in Figure 2.14. These discrepancies can be explained through changes in the protocol:

- 1. At the lower concentrations, the higher percentage of beads remaining in the detection area in Figure 4.9 is a result of dotting the surface of the IC with $1\mu l$ of the goat IgG, rather than coating the entire well. This effectively concentrates the analyte to the surface of the IC, resulting in more specific interactions.
- 2. At the 100ng/ml concentration, only 30% of the beads remained in the center of the trenches in Figure 4.9, as opposed to close to 100% in Figure 2.14. This was due to the fact that 2-fold stronger forces were used to more quickly separate the bead (8 seconds per trench versus 30 seconds). Due to the dearth of chips, only one integrated assay was performed at the 1µg/ml data point and it showed that 90% of the beads remained in the center of the trench, suggesting that the dynamic range of the on-chip protocol extends past 100ng/ml.



Purified Human IgG Assay

Figure 4.8: On-chip purified human IgG assays



Figure 4.9: Relative number of beads remaining in the detection area after magnetic separation



Purified Human IgG Assay

Figure 4.10: Comparison of the on-chip assay with ELISA

4.4.4 On-Chip Dengue Assay Results

Figure 4.11 shows the on-chip assay results for the detection of anti-dengue IgG in Human sera samples. Two dengue positive and two dengue negative sera samples were assayed on-chip and compared to ELISA. The on-chip assay protocol was able to successfully discriminate between the positive and negative sera samples, albeit with reduced signal to noise ratio compared to ELISA. While these initial results are encouraging, the small number of available ICs prohibited on-chip assay protocol optimization and limited more extensive statistically significant bio-chemical selectivity studies.



Dengue Assays

Figure 4.11: On-chip assay on human sera samples

4.5 CMOS Assay Platform Characteristics

The most important characteristic of an effective PON IVD device are high biochemical sensitivity and selectivity, fast operation, low cost, and ease-of-use. The manners in which these issues are fundamentally addressed with this design are discussed below; **Sensitivity** – The high bio-chemical sensitivity displayed by this assay platform is the result of several factors; a) magnetic bead labeling leads to assay sensitivities equivalent to enzymatic labeling for compact, rapid protocols, b) the ability to precisely adjust the separation forces applied on the assay labels results in lower background signal, and c) the ability to detect numerous individual assay labels rather than aggregates statistically reduces background noise. The 700fM detection limit of this platform is superior to conventional ICT, which range from 35pM on purified protein² to 1.6nM on raw samples³, while 2 orders of dynamic range is useful for most applications.

Selectivity – Initial data suggests that the magnetic forces applied to the molecular tethers enables the precise removal of magnetic beads non-specifically or cross-reactively bound to the surface of the chip. In a manufacturing environment, dynamic control of the forces, or the application of a range of forces is useful for achieving a high level of bio-chemical specificity and to mitigate the natural variability in Human sera.

Speed – The surface bio-functionalization can be performed on the factory floor and shall not be accounted in the total PON assay time, which can be broken down into analyte incubation, label incubation, and analyte separation and detection steps. The lengthy analyte incubation step can be minimized by incubating the magnetic beads with the soluble analyte, rather than having the soluble analyte diffuse to the binding sites on the surface of the IC. In such a situation, quasi-solution phase kinetics can be achieved, ideal for PON application. The remaining label incubation, separation and detection steps take under 5 minutes using the on-chip assay protocol.

Ease-of-use – The burden on the user of a digital PON IVD device can be minimized by integrating the assay protocol as much as possible. Ideally, a user would simply present a sample and wait for the results. Stand-alone operation is also an important factor to ensuring ease-of-use since a protocol involving multiple components becomes daunting to the occasional untrained user. The main advantages that stand-alone digital platform have over ICTs with respect to ease of use are a) results are displayed digitally so there is no need for interpretation, b) there is no need for timing, c) instructions can be delivered audio-visually, and d) post-analysis of the assay results can be performed by the device. Figure 4.12 shows the digital display that breaks down final bead tally according to the position in the array. Such a display is necessary for analyzing multiplexed results.

Cost – The use of CMOS minimizes both the development and manufacturing costs. A digital PON IVD tester must have at least one digital IC to drive the display. This system can integrate the various assay components (label separation and detection) on the same chip that is responsible for digitizing and displaying the results. Such a PON IVD implementation would require only one chip, one battery and one display, and therefore would offer a generational low manufacturing cost structure. In terms of development costs, non-recurring costs can be contained by employing legacy technology nodes with standard design flows. These overall cost advantages can be translated into

performance by increasing the effective assay surface area or into added functionality by adding additional types of sensors and/or wireless capabilities.

Added Versatility – Integrated magnetic separation of magnetic bead labels enables sophisticated POC assay modalities. Varying magnetic separation forces can be applied in different trenches on the same chip, and dynamic selection of the optimal force can mitigate variability of the non-specific binding characteristics at the surface of the IC^7 . As an extension of this concept, large control forces can be applied to ensure the appropriate affinity of the specific complementary bonds and therefore validity the assay. Lastly, comprehensive on-chip controls and detection of panels of bio-markers are possible with multiplexed operation²¹.

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Figure 4.12: Digital display of on-chip assay results

4.6 Conclusion

This chapter presented a fully integrated CMOS assay platform that combined onchip magnetic bead separation and detection. The system consisted of 16 trenches lined by separation conductors, with a row of 8 Hall sensor/micro-coil pairs under the center of each trench. The non-specifically bound magnetic beads that sedimented to the surface of the IC were pulled to the side of the trench by 50mA of current flowing though the magnetic separation conductors, which generated 13.2pN of force on the bio-chemical tethers immobilizing the beads. Afterwards, the specifically bound magnetic beads that remained in the center of the trenches were magnetized and detected by the integrated micro-coils and Hall sensors respectively. The magnetic beads were detected with over 95% accuracy, while the assay results showed that concentrations down to 100pg/ml were detectable, and that quantitation was also possible with appropriate on-chip controls. Lastly, assays on Human sera sample suggested that this platform had the biochemical selectivity necessary to distinguish one particular anti-dengue IgG idiotype, although statistical significance was stifled by the limitation in the number of chips. Through previously unachievable levels of integration, this bio-assay platform has the all qualities of an effective PON IVD device, namely high biochemical sensitivity and specificity, speed, ease-of-use, low cost and versatility. The next chapter will present a low-cost easy-to-use membrane filter based sample preparation system that may be combined with this assay platform into a stand-alone digital PON IVD tester.

Chapter 5

5 Sample Preparation and System Integration

5.1 Introduction

An effective PON IVD test must be capable of analyzing unprocessed samples such as whole blood, urine or saliva. Whole blood was selected for this work, since it contains a wider variety of bio-markers, and at higher concentrations than other sample types. For high sensitivity PON IVD applications, a sample preparation system is needed to remove or neutralize the interfering agents such as clotting factors and blood cells. Discrimination of these agents can be performed according to size, surface charge, surface hydrophobicity and affinity or a combination thereof. The on-chip affinity separation presented in Chapter 2 has the ability to distinguish the target analyte from the other soluble proteins, so the requirements on the sample preparation used with this system are greatly reduced.

Apart from soluble proteins, unprocessed whole blood also contains larger 2- $10\mu m$ wide cells such as platelets, hemoglobin and lymphocytes. ICTs use size discrimination to separate these larger species from the small soluble analyte that flow along the thin capillaries.

We present a sample preparation system that traps whole blood cells in a membrane filter, while allowing the soluble analyte to pass. Initial filtration experiments on Human whole blood were used to determine the optimal filter type. Subsequently, its ability to transmit Human IgG was verified by assaying the filtrate using a conventional ELISA, an ELISA on gold and a magnetic bead labeled assay. Lastly, the filtration protocol of Human whole blood spiked with serum from a dengue positive individual is optimized with respect to the amount of anti-dengue antibodies that pass through.

In a subsequent discussion, we show how this system can be readily combined with the CMOS assay platform from the previous chapter in a fully integrated low cost PON bio-assay system.

5.2 System Overview

The sample preparation system consists of a membrane filter fitter to the mouth of vial containing a diluent buffer. Membrane filtration was selected over conventional lateral flow technologies since membranes have higher cross sectional areas and shorter lengths, and can therefore lead to faster sample preparation times. Whole blood from a finger stick is placed on the topside of the membrane, which wicks in the blood and traps the whole blood cells. The device is then turned over 10 times in 30 seconds such that the

soluble protein is eluted through the backside of the filter by the buffer in the vial. Finally, the filtrate containing the target analyte is retrieved and analyzed using one of the various assay protocols. This sample preparation sequence is demonstrated in Figure 5.1.



Figure 5.1: Membrane filter sample preparation system

5.3 System Design

The vial consisted of a 700 μ l micro-centrifuge tube filled with buffer solution. The desirable characteristics of the membrane filter fitted onto that vial are enumerated below:

- 1. The topside of the membrane must have pores with large enough diameter to quickly wick the whole blood.
- 2. The backside of the membrane must have pores with smaller diameter to prevent red blood cells from passing
- 3. The membrane material must be hydrophobic to quickly wick the blood plasma without absorbing protein.
- 4. The membrane must not lyse the red blood cells since doing so would release proteases and other interfering species that could impact the performance of the assay.
- 5. The membrane must be thin to minimize the total amount of whole blood needed.

Table 5.1 lists membrane specifications for different Polyvinylpyrrolidone/Polyethersulfone(PVP/PES) membranes from International Point of Care, Inc (Toronto, Canada) that are tested. The performance of these filters is evaluated according to 1) their ability to wick in whole blood, 2) the amount of whole blood cells that passed through, and 3) the amount of total Human IgG transmitted. The filter with the best overall characteristics is used to filter whole blood spiked with serum from a Dengue positive individual.

Membrane	Average	Porosity	Capacity
Type	Thickness	Gradient	
S/G	260-300 μm with CV of 5%	~ 35 µm (top) ~ .5 µm(bottom)	50 μL of whole blood
NX	210-250 µm with CV of 5%	~ 35 µm (top) ~2.5 µm(bottom)	35 μ L of plasma from 100 of whole blood per cm ² . Depending upon hematocrit
X	160 -200 μm	~ 35 µm (top)	40 μ L of plasma from 110 –
	with CV of 5%	~5 µm(bottom)	120 μ L of whole blood per cm ²
C/Q	130-200 µm with	~ 12 µm (top)	12-16 μ L per cm ² Depending
	CV of 3%	~ 3 µm(bottom)	on membrane thickness
C/S	200-300 µm with CV of 5%	~ 35 μm (top) ~2.5 μm(bottom)	15-22 μ L/cm ² depending on membrane thickness

 Table 5.1: PVP/PES membrane filter specifications according to International Point

 of Care, Inc, Toronto, Canada

5.4 Sample Preparation Protocols

5.4.1 Vial Assembly

The caps of the 700 μ l micro centrifuge tube were cut off and the tubes were filled with 300-400 μ l PBS-Tween diluent buffer. When less than 300 μ l of buffer was added, surface tension prevented the diluent from flowing onto the membrane and back when the vial was overturned. Alternatively, when a larger amount of buffer was added, the dilution factor increased and the concentration of target analyte in the filtrate decreased. The membranes came in large sheets and were cut and placed onto the top of the tube, with the smaller pore size facing down. The combination was sealed along the edge of the mouth of the tube using thin strips of parafilm.

5.4.2 Total Human IgG Filtration

A 30µl drop of fresh whole blood was placed on the topside of the prepared filtration device. The device was then turned over 10 times in 30 seconds such that the diluent buffer in the tube flowed onto the backside of the filter and into the vial containing 400µl of PBS-Tween. After agitation, the filter was carefully removed to prevent any whole blood from entering the tube, and the filtrate was assayed.

5.4.3 Anti-Dengue IgG Filtration

For the dengue experiments, a 25µl drop of fresh whole blood was mixed with 5µl of serum from a dengue positive individual. This 30µl drop of spiked whole blood was

placed on the topside of the prepared filter device. To maximize the amount of antidengue antibodies allowed through the membrane, the time the device was left to sit after application of the blood was varied, as was the number of time the device was overturned. For this experiment, the vial contained 300µl of PBS-Tween

5.4.4 Filtrate Assay Protocols

The ELISA on polystyrene, ELISA on gold and M-280 bead assay on gold protocols used for quantifying the amount of total Human IgG in the filtrate are described in section 2.3, while the Dengue assay protocol used to verify that all the anti-dengue antibodies transmitted through the filter is detailed in section 4.4.2.

5.5 Results

5.5.1 Filter Selection

Figure 5.2 shows the topsides of the S/G, NX, X, C/Q, and C/S PVP/PES membrane filters that were compared for their ability to wick away 30µl of whole blood. The S/G and the X PVP/PES filters performed best since they wicked in all the whole blood. Furthermore, analysis of the centrifuged filtrate demonstrated that the PVP/PES filters were able to block over 99% whole blood cells from passing.

The 30µl of whole blood placed onto the filter contained approximately equal volumes of red blood cells and plasma. Of the 15 µl of serum, 5 µl were absorbed by the filter as specified by the International Point of Care, Inc datasheet, and 10 µl were eluted into the 400 µl of PBS-T in the vial, implying a 1:40 serum dilution in the filtrate. The filtrate was further diluted 1:25, for a total serum dilution of 1:1000. ELISA standard curves were produced from serial 4-fold dilutions starting with 1:1000 for a) a centrifuged Human serum control, b) the filtrate from a small 0.22um pore size Millipore filter (Millipore, Massachusetts, USA), c) the filtrate from a C/S PVP/PES filter, d) the filtrate from a C/Q PVP/PES filter, e) the filtrate from a S/G PVP/PES filter, and f) a PBS negative control. The results, shown in Figure 5.3 suggest that the S/G PVP/PES membrane is best at transmitting IgG. However, the S/G aliquots loose more Human IgG signal at higher dilutions than the unfiltered controls. While not altogether clear, this effect might be due to a small number of interfering agents such as proteases from the few lysed red blood cells that degrade protein at low levels. This hypothesis was reinforced by the slight pink coloration of the filtrate. Less aggressive agitation protocols may be needed to avoid cell lysis.

Overall, the optimal filter was the S/G PVP/PES filter (International Point of Care, Inc); the 35 μ m topside pore size quickly wicked in the whole blood, while the 0.5 μ m backside pore size blocked the whole blood cells from passing. Moreover, assays on the filtrates revealed that the S/G filter transmitted Human IgG most effectively. Figure 5.4 shows a schematic of the cross section of this filter trapping red blood cells.



Figure 5.2: Whole blood wicking characteristics of the S/G, NX, X, C/Q, and C/S PVP/PES membrane filters, from left to right



Whole Blood Filtrates

Figure 5.3: Human IgG assay performed on a) a centrifuged Human serum control, b) a Millipore .22um filtrate (Millipore, MA, USA), c) a C/S PVP/PES membrane filtrate, d) a C/Q PVP/PES membrane filtrate, e) a S/G PVP/PES membrane filtrate, and f) a negative control. All the PVP/PES membranes are from International Point of Care, Inc, Toronto, Canada.




5.5.2 Total Human IgG Assays

To quantify the performance of this filter, serial 10-fold dilutions of the Human whole blood filtrate were assayed using ELISA on polystyrene, ELISA on gold and magnetic bead assay on gold. The corresponding standard curves for total Human IgG are presented in Figure 5.5. Eight experiments were performed for each data point.

The results show that the bead based assays performed best with detection down to $4 \cdot 10^7$ dilutions. The ELISAs on gold perform equally well, when omitting the errant data point at the $4 \cdot 10^8$ dilution. Interestingly, the ELISAs on polystyrene performed much more poorly, as only dilution of $4 \cdot 10^4$ were detectable, consistent with the results from Figure 5.3.

The typical concentration of total IgG in Human whole blood is approximately 5mg/ml³⁰. Therefore, the magnetic bead labeled assays detected Human IgG down to concentrations of 125pg/ml, or 900fM, in line with the results of the control bead labeled assays in Chapter 2, and suggesting that this membrane filtration protocol is capable of quickly and effectively preparing the raw whole blood sample for analysis using the fully integrated assay platform described in the previous Chapter.



Figure 5.5: Whole blood filtrate assay results using ELISA on polystyrene, ELISA on gold and magnetic bead based assays

5.5.3 Anit-Dengue IgG Assays

The amount of time the dengue spike whole blood was allowed to wick through the membrane before the device was overturned was varied to maximize the amount of anti-dengue antibodies that pass through the membrane filter. Figures 5.6 and 5.7 present the ELISA results for a 30-second and a 2-minute wait period respectively. In addition, the number of times the devices was overturned after the waiting period was varied. The best results, presented in Figure 5.6, were achieved with a 2 minute wait time followed by 10 turns in 30 seconds. The 5 μ l of dengue positive serum were filtered into 300 μ l of PBS-T buffer. Assuming a loss of 33% of the protein into the filter as specified by the International Point of Care, Inc datasheet, the final dengue serum dilution in the filtrate was 1:100. In these experiments, the positive control was also a 1:100 dilution of dengue positive Human serum in PBS-T. We can therefore conclude that by comparison, approximately 66% of the anti-dengue antibodies were transmitted through the filter.



Figure 5.6: Assay on spiked whole blood filtrate with a 30 seconds wait time between application of the blood and agitation



Figure 5.7: Assay on spiked whole blood filtrate with a 2 minutes wait time between application of the blood and agitation

5.6 Discussion

5.6.1 Integration with the CMOS-based Bio-Assay Platform

This membrane filtration system can be combined with the fully integrated assay platform in an arrangement depicted in Figure 5.8. A drop of whole blood is placed on the membrane filter, and the top of the vial is sealed. The cartridge is then agitated to elute the protein through the filter and into the buffer solution in the vial. This agitation concurrently re-suspends in solution the magnetic beads found in the vial, which bind to the target analyte and which then settle via gravity to the surface of the IC bonded facing up at the bottom of the vial. The target analyte strongly tethers the magnetic beads to the surface of the IC through a strong immunological complex. The non-immunologically bound beads are removed by on-chip generated magnetic forces, while the immunologically bound beads remain stationary and are detected by an array of Hall sensors embedded under the surface of the IC.

This scheme is ideal for reducing incubation times since solution phase kinetics can be achieved by capturing the target analyte in the filtrate with the re-suspended magnetic beads. Lengthy diffusion times are overcome by the beads settling quickly via gravity to the surface of the chip, and the total analyte incubation time can be modulated through the bead settling distance.



Figure 5.8: Integration of the membrane filtration with the CMOS bio-Assay platform

5.6.2 Stand-Alone Digital PON IVD Tester

The subsystem containing the sample preparation and the CMOS assay platform can be combined with a battery and a display into a low-cost, easy to use PON IVD tester presented in Figure 5.9. This device would be digital to avoid ambiguous reporting, highly accurate to detect early stage conditions and quantitative for more controlled treatment options. Ideally, patients would purchase this device over the counter and self administer diagnoses for a variety of diseases ranging from cancer to pregnancy to infectious diseases. Doctors and public health officials can be notified wirelessly of conditions that require immediate action or that have societal implications.



Figure 5.9: Cross-section of stand-alone digital PON IVD tester

5.6.3 Future Directions

While the initial results are encouraging, statistical significance and repeatability work were restricted due to the limited number of CMOS chips available for this work. While the sensitivity displayed by this assay platform was high, the quantitative resolution was lacking. To overcome the high variability that degraded quantitative precision, the number of sensors can be increased to over 1000, on-chip concentration as described in Chapter 2 can be implemented on the assay platform to further increase the signal amplitude, and the surface functionalization can be improved through the use of hetero-bifunctional cross-linkers that bind and orient the antibodies densely on the gold surface³².

Increasing the number of sensors is troublesome due to the increased power dissipation as the magnetic separation conductors get longer. To mitigate this effect, the trenches can be narrowed and the detection range of the Hall sensors modulated accordingly. This way, less power is needed to remove the magnetic beads from atop the Hall sensor.

The specificity of this assay platform has been only cursorily verified using dengue positive Human serum samples. Historically however, as with Aytur et al.¹⁹, high bio-chemical sensitivity for the detection of purified samples has translated well for the detection of highly diverse Human serum samples. The easy fine tuning of the magnetic separation forces will be invaluable in developing a protocol appropriate for assaying unprocessed Human samples.

Integration of the back-end electronics on-chip is essential for reducing the cost of the stand-alone Digital PON IVD tester. This involves integrating the digitization and all the corresponding external controls. Fortunately, the frequency of operation of the IC is in the range of 50kHz-5MHz, which makes on-chip integration of all these components relatively straight-forward.

Finally, much work remains on protocol integration; the filtration protocol must be seamlessly combined in a robust repeatable manner with the on-chip assay protocol as described in section 5.6.1.

5.7 Conclusion

In this chapter we have demonstrated a low cost, easy to use sample preparation system that relies on membrane filtration to block agents such as red blood cells that may interfere with a bio-chemical assay, and to allow the target analyte to pass. We have tested a variety of filters concluded that the S/G PVP/PES filter (International Point of Care, Inc) performed best due to its ability to wick blood, trap red blood cells and elute the target protein. Magnetic bead labeled assays suggest that human IgG in the filtrate is detectable down to 125pg/ml, or 900fM. Lastly, whole blood was spiked with serum taken from a dengue positive individual, and the mixture was filtered. ELISAs used to optimize the filtration protocol showed that approximately 66% of the anti-dengue

antibodies presented on the top of the filter were retrieved in the filtrate. This effective sample preparation technology can be integrated with the fully integrated CMOS bioassay platform presented in the previous Chapter in a single-chip PON IVD tester solution. Such a low cost, high functionality platform can enable a variety of new applications in developing nations and in emerging fields like telemedicine. Even more broadly however, this technology has the potential to decentralize medical care, and by so doing release the creative energy that has been restrained by the structural rigidities of that sector.

Appendix

NI-6259 C-Code for Control of the Fully Integrated Assay Platform

```
#include <stdio.h>
#include <malloc.h>
#include <string.h>
#include <NIDAQmx.h>
#include <time.h>
#include <math.h>
#define DAQmxErrChk(functionCall) if( DAQmxFailed(error=(functionCall))
) goto Error; else
#define PI 3.1415926535
int32 CVICALLBACK DoneCallback(TaskHandle taskHandle, int32 status,
void *callbackData);
float64 calibrate(float64 ao cal values[], float64 cal amplitude[],
FILE * LogFile);
void detect(float64 ao cal values[], float64 bead amplitude[], FILE *
LogFile);
void wash(float64 wash voltage, int cycles, FILE * LogFile);
void concentrate(void);
void main(void)
{
      float64 ao cal values[128];
      float64 cal amplitude[128];
      float64 bead amplitude[128];
      float64 CDS[128];
      int number of beads = 0;
      int i,j,k;
      FILE * LogFile;
      FILE * CalFile;
      float64 wash voltage=0.0;
      int cycles=1;
      int scan;
      int addr;
      float64 ao value;
      float64 amplitude;
      int read status = 0;
      int mode;
      int ChipID = 0;
      printf("Please make a selection:\n");
      printf("1. Calibrate\n2. Detect\n");
      scanf("%i", &mode);
      getchar();
      if (mode==1)
      {
```

```
LogFile = fopen("c:\\Documents and
      Settings\\General\\Desktop\\AssayLogs\\calibrate log.txt","
      w");
      CalFile = fopen("c:\\Documents and
      Settings\\General\\Desktop\\AssayLogs\\cal.txt","w");
      printf("Calibtrating...\n");
      fprintf(LogFile, "Calibtrating...\n");
      calibrate(ao cal values, cal amplitude, LogFile);
      detect(ao cal values, cal amplitude, LogFile);
      for (i=0; i<=127;++i)</pre>
      {
            fprintf(CalFile,"%i\n", i);
            fprintf(CalFile,"%lf\n",ao_cal_values[i]);
            fprintf(CalFile,"%lf\n",cal amplitude[i]);
      }
}
else if (mode==2)
{
      LogFile = fopen("c:\\Documents and
      Settings\\General\\Desktop\\AssayLogs\\detect log.txt","w")
      CalFile = fopen("c:\\Documents and
      Settings\\General\\Desktop\\AssayLogs\\cal.txt","r");
      for (i=0; i<=127; ++i)</pre>
      {
            scan=fscanf(CalFile,"%i", &addr);
            scan=fscanf(CalFile,"%lf",&ao value);
            scan=fscanf(CalFile,"%lf",&amplitude);
            ao cal values[addr]=ao value;
            cal amplitude[addr]=amplitude;
      }
      for (i=0; i<=127;++i)</pre>
      {
            printf("Address: %i\tAO cal: %f\tSig:
            %f\n",i,ao cal values[i], cal amplitude[i]);
            fprintf(LogFile, "Address: %i\tAO cal: %f\tSig:
            %f\n",i,ao cal values[i], cal amplitude[i]);
      }
      while (cycles > 0)
      {
            printf("Enter the number of washing cycles:\t");
            scanf("%i",&cycles);
            getchar();
            printf("Enter the washing voltage:\t");
            scanf("%lf",&wash voltage);
            getchar();
            printf("Washing for %i cycles at %lf volts\n",
            cycles, wash voltage);
            fprintf(LogFile, "Washing for %i cycles at %lf
            volts\n", cycles, wash voltage);
            wash(wash voltage,cycles, LogFile);
      }
```

```
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```

```
printf("Detecting...\n");
      fprintf(LogFile, "Detecting...\n");
      detect(ao cal values, bead amplitude, LogFile);
      printf("CDS Signal\n");
      fprintf(LogFile, "CDS Signal\n");
      for(i=0;i<=127;++i)</pre>
      {
            CDS[i]=bead_amplitude[i]-cal_amplitude[i];
            printf("Address: %i\tCal: %f\tSig: %f\tCDS:
            %f\n",i,cal amplitude[i], bead amplitude[i],CDS[i]);
            fprintf(LogFile, "Address: %i\tCal: %f\tSig: %f\tCDS:
            %f\n",i,cal_amplitude[i], bead_amplitude[i],CDS[i]);
            fprintf(CalFile,"%i\n%f\n%f\n",i,ao cal values[i],
            bead amplitude[i]);
            if (fabs(CDS[i])>0.001)
                   ++number of beads;
      }
      printf("\n\nTotal Number of Beads Detected:
      %i\n\n",number of beads);
      fprintf(LogFile,"\n\nTotal Number of Beads Detected:
      %i\n\n",number of beads);
      for (i=0; i<=7; ++i)</pre>
      {
            for (j=0; j<16; ++j)</pre>
            {
                   k=((j+1)%2)*(i+8*(j))+(j%2)*((15-i)+8*(j-1));
                   if (fabs(CDS[k])>0.001)
                   {
                         printf("| x ");
                         fprintf(LogFile,"| x ");
                   }
                   else
                   {
                         printf("| 0 ");
                         fprintf(LogFile,"| 0 ");
                   }
            }
            printf("|\n");
            fprintf(LogFile,"|\n");
      }
else
      printf("Incorrect Selection. Goodbye.\n");
      getchar();
printf("\nPress enter to finish");
getchar();
fclose (LogFile);
fclose (CalFile);
return;
```

```
69
```

}

{

}

}

```
11
11
     void detect(cal amplitude[])
11
11
     This function detects the presence of beads
11
11
    Hardware Settings:
11
        ao0 - I_ref
11
          aol - I cancel
11
          Dev1/port2/line4 - clock
11
          Dev1/port2/line6 - data
11
          ai0 - Vh+
11
          ai8 - Vh-
11
void detect(float64 ao cal values[], float64 bead amplitude[], FILE *
LogFile)
{
     float64*
               data = malloc(sizeof(float64)*240000);
                     cosine = malloc(sizeof(double)*960000);
     double*
     double
                     val[100];
                     amplitude=1;
     double
     int32
              error=0;
     TaskHandle taskHandle=0;
     TaskHandle taskHandle2=0;
     TaskHandle taskHandle3=0;
TaskHandle taskHandle4=0;
TaskHandle taskHandle5=0;
     uInt8 data1[16]={0,0,0,1,1,0,0,1,1,0,0,0,0,0,0,0};
     uInt8
              dataWrite[8]={0,0,0,0,0,0,0,0;;
              errBuff[2048]={'\0'};
     char
              dataRead[100];
     uInt8
     int32
               i,j,k,l,m,n;
     int
                     address;
                read,bytesPerSamp;
     int32
     float64
                     ao ref value=0.7;
     // Initialize square[]
     // 1,200,000 sample/second
     // 120,000 samples (1/10 seconds)
     // 50kHz signal
     // 5,000 cycles in 1/10 seconds
     // 24 samples per cycle
     for(i=0;i<9600;++i)</pre>
     {
          for (j=0; j<100; ++j)</pre>
           {
                cosine[i*100+j]=cos(j*2*PI/100);
           }
     }
```

```
// Initialize val[]
/****
               ****
     * * * * * * * *
for (j=0; j<100; ++j)</pre>
{
    val[j]=0;
}
// DAQmx digital write
DAQmxErrChk (DAQmxCreateTask("",&taskHandle));
DAQmxErrChk
(DAQmxCreateDOChan(taskHandle,"Dev1/port2/line0:7","",DAQmx Val C
hanForAllLines));
// DAQmx clock turned on to excite the beads
DAQmxErrChk (DAQmxCreateTask("", &taskHandle2));
DAOmxErrChk
(DAQmxCreateCOPulseChanFreq(taskHandle2, "Dev1/ctr0", "", DAQmx Val
Hz, DAQmx Val Low, 0.0, 100000, 0.50));
DAOmxErrChk
(DAQmxCfgImplicitTiming(taskHandle2,DAQmx Val ContSamps,1000));
DAQmxErrChk
(DAQmxRegisterDoneEvent(taskHandle2,0,DoneCallback,NULL));
// Set analog value on Ref current to nominal value
/*****************
                         * * * * * * * * * * * * * /
DAQmxErrChk (DAQmxCreateTask("",&taskHandle5));
DAQmxErrChk
(DAQmxCreateAOVoltageChan(taskHandle5,"Dev1/ao0","",0.0,3,DAQmx V
al Volts, NULL));
DAQmxErrChk
(DAQmxRegisterDoneEvent(taskHandle5,0,DoneCallback,NULL));
DAQmxErrChk (DAQmxStartTask(taskHandle5));
DAQmxErrChk (DAQmxWriteAnaloqScalarF64(taskHandle5, 0, 10.0, 0,
NULL));
// Set analog value on Cancel current to nominal value
DAQmxErrChk (DAQmxCreateTask("",&taskHandle3));
DAQmxErrChk
(DAQmxCreateAOVoltageChan(taskHandle3,"Dev1/ao1","",0.0,3,DAQmx V
al Volts, NULL));
DAQmxErrChk
(DAQmxRegisterDoneEvent(taskHandle3,0,DoneCallback,NULL));
DAQmxErrChk (DAQmxStartTask(taskHandle3));
DAQmxErrChk (DAQmxWriteAnalogScalarF64(taskHandle3, 0, 10.0, 0,
NULL));
```

```
// Analog Read
```

```
DAQmxErrChk (DAQmxCreateTask("", &taskHandle4));
DAQmxErrChk
(DAQmxCreateAIVoltageChan(taskHandle4, "Dev1/ai0", "Diff input", DAQ
mx Val Diff,-0.2,0.2,DAQmx Val Volts,NULL));
DAQmxErrChk
(DAQmxCfgSampClkTiming(taskHandle4,"",1200000.0,DAQmx Val Rising,
DAQmx Val FiniteSamps, 240000));
for (n=0; n \le 130; ++n)
{
     l=(17*n)%128;
     //l=n%128;
     for (m = 0; m \le 6; ++m)
     {
          data1[9+m] = (1 >> m) \& 0x01;
     }
     DAQmxErrChk (DAQmxStartTask(taskHandle));
     // Clear the data buffer dataWrite[6] by toggling
     dataWrite[4] 32x
     // corresponding to Dev1/port2/line6 and 4 respectively
     for (j=0; j<32;++j)</pre>
     {
          dataWrite[6] = 0;
          dataWrite[4] = 0;
          DAQmxErrChk
          (DAQmxWriteDigitalLines(taskHandle,1,1,10.0,DAQmx Val
           GroupByChannel,dataWrite,NULL,NULL));
          DAQmxErrChk
          (DAQmxReadDigitalLines(taskHandle,1,10.0,DAQmx Val Gr
          oupByChannel,dataRead,100,&read,&bytesPerSamp,NULL));
          dataWrite[4] = 1;
          DAOmxErrChk
          (DAQmxWriteDigitalLines(taskHandle,1,1,10.0,DAQmx Val
           GroupByChannel,dataWrite,NULL,NULL));
          DAOmxErrChk
          (DAQmxReadDigitalLines(taskHandle,1,10.0,DAQmx Val Gr
          oupByChannel,dataRead,100,&read,&bytesPerSamp,NULL));
     }
     // Uploading data from data1[16] starting with a leading 1
     dataWrite[6] = 1;
     dataWrite[4] = 0;
     DAQmxErrChk
     (DAQmxWriteDigitalLines(taskHandle,1,1,10.0,DAQmx Val Group
     ByChannel,dataWrite,NULL,NULL));
     DAQmxErrChk
     (DAQmxReadDigitalLines(taskHandle,1,10.0,DAQmx Val GroupByC
     hannel,dataRead,100,&read,&bytesPerSamp,NULL));
```

```
dataWrite[4] = 1;
DAQmxErrChk
(DAQmxWriteDigitalLines(taskHandle,1,1,10.0,DAQmx Val Group
ByChannel,dataWrite,NULL,NULL));
DAQmxErrChk
(DAQmxReadDigitalLines(taskHandle,1,10.0,DAQmx Val GroupByC
hannel,dataRead,100,&read,&bytesPerSamp,NULL));
for (j=0; j<16; ++j)</pre>
{
      dataWrite[6] = data1[j];
      dataWrite[4] = 0;
      DAQmxErrChk
      (DAQmxWriteDigitalLines(taskHandle,1,1,10.0,DAQmx Val
      GroupByChannel,dataWrite,NULL,NULL));
      DAQmxErrChk
      (DAQmxReadDigitalLines(taskHandle,1,10.0,DAQmx Val Gr
      oupByChannel,dataRead,100,&read,&bytesPerSamp,NULL));
      dataWrite[4] = 1;
      DAQmxErrChk
      (DAQmxWriteDigitalLines(taskHandle,1,1,10.0,DAQmx Val
      GroupByChannel,dataWrite,NULL,NULL));
      DAOmxErrChk
      (DAQmxReadDigitalLines(taskHandle,1,10.0, DAQmx Val Gr
      oupByChannel,dataRead,100,&read,&bytesPerSamp,NULL));
}
dataWrite[4] = 0;
dataWrite[6] = 0;
DAQmxErrChk
(DAQmxWriteDigitalLines(taskHandle,1,1,10.0,DAQmx Val Group
ByChannel,dataWrite,NULL,NULL));
DAQmxErrChk
(DAQmxReadDigitalLines(taskHandle,1,10.0,DAQmx Val GroupByC
hannel,dataRead,100,&read,&bytesPerSamp,NULL));
DAQmxStopTask(taskHandle);
DAQmxErrChk (DAQmxStartTask(taskHandle2));
DAQmxErrChk (DAQmxWriteAnalogScalarF64(taskHandle3, 0,
10.0, ao cal values[1], NULL));
DAQmxErrChk (DAQmxWriteAnalogScalarF64(taskHandle5, 0,
10.0, ao ref value, NULL));
DAQmxErrChk (DAQmxStartTask(taskHandle4));
DAOmxErrChk
(DAQmxReadAnalogF64(taskHandle4,240000,10.0,DAQmx Val Group
ByScanNumber, data, 240000, &read, NULL));
//DAQmxErrChk (DAQmxWriteAnalogScalarF64(taskHandle3, 0,
10.0, 0, NULL));
//DAQmxErrChk (DAQmxWriteAnalogScalarF64(taskHandle5, 0,
10.0, 0, NULL));
DAQmxStopTask(taskHandle2);
DAQmxStopTask(taskHandle4);
```

```
// Process signal
           amplitude=0;
           for (j=0; j<100; ++j)</pre>
                 val[j]=0;
           for (i=0; i<240000; ++i)</pre>
           {
                 for (j=0; j<100; ++j)</pre>
                      val[j]+=cosine[4*i+j]*data[i];
           }
                 for (j=0; j<100; ++j)</pre>
           {
                 if (fabs(val[j])>amplitude)
                      amplitude=fabs(val[j]);
           }
           amplitude=amplitude/120000;
           printf("Address: %i\tAmplitude: %f\tCoil Value:
           %f\n",l,amplitude, ao cal values[l]);
           fprintf(LogFile,"Address: %i\tAmplitude: %f\tCoil Value:
           %f\n",1,amplitude, ao cal values[1]);
           bead amplitude[l]=amplitude;
     DAQmxErrChk (DAQmxWriteAnalogScalarF64(taskHandle3, 0, 10.0, 0,
     NULL));
     DAQmxErrChk (DAQmxWriteAnalogScalarF64(taskHandle5, 0, 10.0, 0,
     NULL));
     DAQmxClearTask(taskHandle);
     DAQmxClearTask(taskHandle2);
     DAQmxStopTask(taskHandle3);
     DAQmxClearTask(taskHandle3);
     DAQmxClearTask(taskHandle4);
     DAQmxStopTask(taskHandle5);
     DAQmxClearTask(taskHandle5);
Error:
     if( DAQmxFailed(error) )
           DAQmxGetExtendedErrorInfo(errBuff, 2048);
     if( taskHandle!=0 ) {
     // DAQmx Stop Code
     DAQmxStopTask(taskHandle);
           DAQmxClearTask(taskHandle);
           DAQmxStopTask(taskHandle2);
           DAQmxClearTask(taskHandle2);
           DAQmxStopTask(taskHandle3);
           DAQmxClearTask(taskHandle3);
           DAQmxStopTask(taskHandle4);
           DAQmxClearTask(taskHandle4);
           DAQmxStopTask(taskHandle5);
           DAQmxClearTask(taskHandle5);
     }
     if( DAQmxFailed(error) )
     {
```

```
printf("DAQmx Error: %s\n",errBuff);
          getchar();
     }
     return;
return;
}
11
11
     void calibrate(cal amplitude[])
11
11
    This function zeroes out the output of each
11
    Hall sensor before the beads are applied.
11
11
   Hardware Settings:
11
         ao0 - I ref
          aol - I cancel
11
11
          Dev1/port2/line4 - clock
11
         Dev1/port2/line6 - data
11
          ai0 - Vh+
11
          ai8 - Vh-
11
float64 calibrate(float64 ao cal values[], float64 bead amplitude[],
FILE * LogFile)
{
     float64* data = malloc(sizeof(float64)*24000);
     double* cosine = malloc(sizeof(double)*96000);
                    val[100];
     double
     double
                    amplitude=0;
                    min_amplitude=2;
     double
     float64 min
int32 error=0;
                    min cal=10;
     TaskHandle taskHandle=0;
     TaskHandle taskHandle2=0;
     TaskHandle taskHandle3=0;
     TaskHandle taskHandle4=0;
     TaskHandle taskHandle5=0;
     uInt8 data1[16]={0,0,0,1,1,0,0,1,1,0,0,0,0,0,0,0};
     uInt8 dataWrite[8]={0,0,0,0,0,0,0,0};
char errBuff[2048]={'\0'};
uInt8 dataRead[100];
int32 i,j,k,1,m,n;
     int
                     adbdress;
     int32 read, bytesPerSamp;
     float64
                ao ref value=0.7;
     float64
                    ao cal value=0.7;
                    step=0.0005;
     float64
     // Initialize square[]
     // 1,200,000 sample/second
     // 120,000 samples (1/10 seconds)
     // 50kHz signal
     // 5,000 cycles in 1/10 seconds
     // 24 samples per cycle
```

```
for (i=0; i<960; ++i)</pre>
{
    for (j=0; j<100; ++j)</pre>
    {
         cosine[i*100+j]=cos(j*2*PI/100);
    }
}
// Initialize val[]
for (j=0; j<100; ++j)</pre>
{
    val[j]=0;
}
// DAQmx digital write
DAQmxErrChk (DAQmxCreateTask("", &taskHandle));
DAQmxErrChk
(DAQmxCreateDOChan(taskHandle, "Dev1/port2/line0:7", "", DAQmx Val C
hanForAllLines));
// DAQmx clock turned on to excite the beads
DAQmxErrChk (DAQmxCreateTask("",&taskHandle2));
DAQmxErrChk
(DAQmxCreateCOPulseChanFreq(taskHandle2, "Dev1/ctr0", "", DAQmx Val
Hz,DAQmx Val Low,0.0,100000,0.50));
DAQmxErrChk
(DAQmxCfgImplicitTiming(taskHandle2,DAQmx Val ContSamps,1000));
DAQmxErrChk
(DAQmxRegisterDoneEvent(taskHandle2,0,DoneCallback,NULL));
// Set analog value on Cancel current to zero
DAQmxErrChk (DAQmxCreateTask("", &taskHandle3));
DAQmxErrChk
(DAQmxCreateAOVoltageChan(taskHandle3, "Dev1/ao1", "", 0.0, 3, DAQmx V
al Volts, NULL));
DAQmxErrChk
(DAQmxRegisterDoneEvent(taskHandle3,0,DoneCallback,NULL));
DAQmxErrChk (DAQmxStartTask(taskHandle3));
DAQmxErrChk (DAQmxWriteAnalogScalarF64(taskHandle3, 0, 10.0, 0.0,
NULL));
// Set analog value on Ref current to zero
DAQmxErrChk (DAQmxCreateTask("", &taskHandle5));
DAOmxErrChk
(DAQmxCreateAOVoltageChan(taskHandle5, "Dev1/ao0", "", 0.0, 3, DAQmx V
al Volts, NULL));
```

```
DAQmxErrChk
(DAQmxRegisterDoneEvent(taskHandle5,0,DoneCallback,NULL));
DAQmxErrChk (DAQmxStartTask(taskHandle5));
DAQmxErrChk (DAQmxWriteAnalogScalarF64(taskHandle5, 0, 10.0, 0.0,
NULL));
// Analog Read
DAQmxErrChk (DAQmxCreateTask("",&taskHandle4));
DAQmxErrChk
(DAQmxCreateAIVoltageChan(taskHandle4,"Dev1/ai0","Diff input",DAQ
mx Val Diff,-0.2,0.2,DAQmx Val Volts,NULL));
DAOmxErrChk
(DAQmxCfgSampClkTiming(taskHandle4,"",1200000.0,DAQmx Val Rising,
DAQmx Val FiniteSamps, 24000));
for (n=0; n <= 135; ++n)
{
     l=(17*n)%128;
     //l=n%128;
     for (m = 0; m<=6; ++m)</pre>
     {
          data1[9+m] = (1 >> m) \& 0x01;
     }
     ao cal value=0.75;
     step=0.05;
     DAQmxErrChk (DAQmxStartTask(taskHandle));
     // Clear the data buffer dataWrite[6] by toggling
     dataWrite[4] 32x
     // corresponding to Dev1/port2/line6 and 4 respectively
     for (j=0; j<32;++j)</pre>
     {
          dataWrite[6] = 0;
          dataWrite[4] = 0;
          DAQmxErrChk
          (DAQmxWriteDigitalLines(taskHandle,1,1,10.0,DAQmx Val
          GroupByChannel,dataWrite,NULL,NULL));
          //DAQmxErrChk
          (DAQmxReadDigitalLines(taskHandle,1,10.0,DAQmx Val Gr
          oupByChannel,dataRead,100,&read,&bytesPerSamp,NULL));
          dataWrite[4] = 1;
          DAQmxErrChk
          (DAQmxWriteDigitalLines(taskHandle,1,1,10.0,DAQmx Val
           GroupByChannel,dataWrite,NULL,NULL));
          //DAQmxErrChk
          (DAQmxReadDigitalLines(taskHandle,1,10.0, DAQmx Val Gr
          oupByChannel,dataRead,100,&read,&bytesPerSamp,NULL));
     }
```

```
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```

```
// Uploading data from data1[16] starting with a leading 1
/****
                                     * * * * * * * * * * /
dataWrite[6] = 1;
dataWrite[4] = 0;
DAQmxErrChk
(DAQmxWriteDigitalLines(taskHandle,1,1,10.0,DAQmx Val Group
ByChannel,dataWrite,NULL,NULL));
//DAQmxErrChk
(DAQmxReadDigitalLines(taskHandle,1,10.0,DAQmx Val GroupByC
hannel, dataRead, 100, & read, & bytesPerSamp, NULL));
dataWrite[4] = 1;
DAQmxErrChk
(DAQmxWriteDigitalLines(taskHandle,1,1,10.0,DAQmx Val Group
ByChannel,dataWrite,NULL,NULL));
//DAQmxErrChk
(DAQmxReadDigitalLines(taskHandle,1,10.0,DAQmx Val GroupByC
hannel,dataRead,100,&read,&bytesPerSamp,NULL));
for (j=0; j<16; ++j)</pre>
{
      dataWrite[6] = data1[j];
      dataWrite[4] = 0;
      DAOmxErrChk
      (DAQmxWriteDigitalLines(taskHandle,1,1,10.0,DAQmx Val
      GroupByChannel,dataWrite,NULL,NULL));
      //DAQmxErrChk
      (DAQmxReadDigitalLines(taskHandle,1,10.0,DAQmx Val Gr
      oupByChannel,dataRead,100,&read,&bytesPerSamp,NULL));
      dataWrite[4] = 1;
      DAQmxErrChk
      (DAQmxWriteDigitalLines(taskHandle,1,1,10.0,DAQmx Val
      GroupByChannel,dataWrite,NULL,NULL));
      //DAQmxErrChk
      (DAQmxReadDigitalLines(taskHandle,1,10.0,DAQmx Val Gr
      oupByChannel,dataRead,100,&read,&bytesPerSamp,NULL));
}
dataWrite[4] = 0;
dataWrite[6] = 0;
DAOmxErrChk
(DAQmxWriteDigitalLines(taskHandle,1,1,10.0, DAQmx Val Group
ByChannel,dataWrite,NULL,NULL));
11
            DAQmxErrChk
(DAQmxReadDigitalLines(taskHandle,1,10.0,DAQmx Val GroupByC
hannel,dataRead,100,&read,&bytesPerSamp,NULL));
DAQmxErrChk (DAQmxWriteAnalogScalarF64(taskHandle5, 0,
10.0, ao ref value, NULL));
DAQmxErrChk (DAQmxWriteAnalogScalarF64(taskHandle3, 0,
10.0, ao cal value, NULL));
DAQmxErrChk (DAQmxStopTask(taskHandle));
DAQmxErrChk (DAQmxStartTask
(taskHandle2));
min cal=10;
amplitude=1;
```

```
min amplitude=2;
while ((amplitude > 0.0002)&&(ao cal value>0.4))
{
      amplitude=0;
      for (j=0; j<100; ++j)</pre>
      val[j]=0;
      DAQmxErrChk (DAQmxStartTask(taskHandle4));
      DAQmxErrChk
      (DAQmxReadAnalogF64(taskHandle4,24000,10.0,DAQmx Val
      GroupByScanNumber, data, 24000, &read, NULL));
      for (i=0; i<24000; ++i)</pre>
      {
            for (j=0; j<100; ++j)</pre>
                   val[j]+=cosine[4*i+j]*data[i];
      }
      for (j=0; j<100; ++j)</pre>
      {
            if (fabs(val[j])/12000>amplitude)
                   amplitude=fabs(val[j])/12000;
      }
      if (amplitude>(min amplitude+0.0010))
      {
            printf("Break - coil value: %f amp: %f max amp:
            %f\n", ao_cal_value, amplitude, min_amplitude);
            fprintf(LogFile, "Break - coil value: %f amp:
            %f max amp: %f\n", ao_cal_value, amplitude,
            min amplitude);
            DAQmxErrChk (DAQmxStopTask(taskHandle4));
            break;
      }
      if (amplitude<min amplitude)</pre>
      {
            min amplitude=amplitude;
            min cal=ao cal value;
      }
      printf("Address: %i%i%i%i%i%i%i%i\tAmplitude: %f\tCoil
      Value: %f\n", data1[15], data1[14], data1[13],
      data1[12],
      data1[11], data1[10], data1[9],
      amplitude,ao cal value);
      fprintf(LogFile, "Address: %i%i%i%i%i%i%i%i%i\tAmplitude:
      %f\tCoil Value: %f\n",data1[15], data1[14],
      data1[13], data1[12], data1[11], data1[10], data1[9],
      amplitude,ao cal value);
      if (amplitude>0.006)
            step=0.08;
      else if (amplitude>0.003)
            step=0.04;
```

```
else if (amplitude>0.002)
                      step=0.008;
                 else if (amplitude>0.0005)
                      step=0.004;
                 else
                      step=0.0008;
                 ao cal value-=step;
                 DAQmxErrChk (DAQmxWriteAnalogScalarF64(taskHandle3,
                 0, 10.0, ao cal value, NULL));
                 DAQmxErrChk (DAQmxStopTask(taskHandle4));
           }
           DAQmxErrChk (DAQmxWriteAnalogScalarF64(taskHandle5, 0,
           10.0, 0, NULL));
           DAQmxErrChk (DAQmxWriteAnalogScalarF64(taskHandle3, 0,
           10.0, 0, NULL));
           DAQmxStopTask(taskHandle2);
           bead amplitude[l]=min amplitude;
           ao cal values[1]=min cal;
     }
     DAQmxClearTask(taskHandle);
     DAQmxClearTask(taskHandle2);
     DAQmxStopTask(taskHandle3);
     DAQmxClearTask(taskHandle3);
     DAQmxStopTask(taskHandle4);
     DAQmxClearTask(taskHandle4);
     DAQmxStopTask(taskHandle5);
     DAQmxClearTask(taskHandle5);
Error:
     if( DAQmxFailed(error) )
           DAQmxGetExtendedErrorInfo(errBuff, 2048);
     if( taskHandle!=0 ) {
     // DAQmx Stop Code
     DAQmxStopTask(taskHandle);
           DAQmxClearTask(taskHandle);
           DAQmxStopTask(taskHandle2);
           DAQmxClearTask(taskHandle2);
           DAQmxStopTask(taskHandle3);
           DAQmxClearTask(taskHandle3);
           DAQmxStopTask(taskHandle4);
           DAQmxClearTask(taskHandle4);
           DAQmxStopTask(taskHandle5);
           DAQmxClearTask(taskHandle5);
     if( DAQmxFailed(error) )
```

```
{
          printf("DAQmx Error: %s\n",errBuff);
          getchar();
     }
     return ao cal value;
return ao cal value;
}
11
11
    void wash()
11
11
    This function washes the non-specifically
11
    bound beads
11
11
   Hardware Settings:
11
        ao0 - I ref
11
         aol - I cancel
11
         Dev1/port2/line4 - clock
11
         Dev1/port2/line6 - data
11
         ai0 - Vh+
11
         ai8 - Vh-
11
void wash(float64 wash voltage, int cycles, FILE * LogFile)
{
    int32 error=0;
TaskHandle taskHandle=0;
TaskHandle taskHandle3=0;
     int32
     TaskHandle taskHandle5=0;
    uInt8 dataWrite[8]={0,0,0,0,0,0,0,0};
             errBuff[2048]={'\0'};
    char
             dataRead[100];
    uInt8
              i,j,k,l,m;
    int32
    audress;
int32 read,bytesPerSamp;
float64 ao rof
float64
              ao ref value=0.0;
           float64
    uInt8
    uInt8
                    start tick;
     clock t
     // Set analog channels
     11
     11
          Turn off currents
     DAQmxErrChk (DAQmxCreateTask("",&taskHandle5));
     DAQmxErrChk
     (DAQmxCreateAOVoltageChan(taskHandle5, "Dev1/ao1", "", 0.0, 3, DAQmx V
     al Volts, NULL));
     DAQmxErrChk
     (DAQmxRegisterDoneEvent(taskHandle5,0,DoneCallback,NULL));
     DAQmxErrChk (DAQmxStartTask(taskHandle5));
```

```
DAQmxErrChk (DAQmxWriteAnalogScalarF64(taskHandle5, 0, 10.0,
ao cal value, NULL));
DAQmxErrChk (DAQmxCreateTask("", &taskHandle3));
DAOmxErrChk
(DAQmxCreateAOVoltageChan(taskHandle3, "Dev1/ao0", "", 0.0, 3, DAQmx V
al Volts, NULL));
DAQmxErrChk
(DAQmxRegisterDoneEvent(taskHandle3,0,DoneCallback,NULL));
DAQmxErrChk (DAQmxStartTask(taskHandle3));
DAQmxErrChk (DAQmxWriteAnalogScalarF64(taskHandle3, 0, 10.0,
ao ref value, NULL));
// Cycle through addresses
// Wash cntrl B=000, down=1, up=0, en clk div2=1, Bypass=0,
en0=1, en1=0
// Note: Clock must be low during washing to re-route the current
in the coils!
// dataWrite[4] is clock
// dataWrite[6] is data
DAQmxErrChk (DAQmxCreateTask("", &taskHandle));
DAQmxErrChk
(DAQmxCreateDOChan(taskHandle,"Dev1/port2/line0:7","",DAQmx Val C
hanForAllLines));
DAQmxErrChk (DAQmxStartTask(taskHandle));
for(i=0;i<cycles;++i)</pre>
{
     for (l=0;l<16;++1)</pre>
     {
           for (m = 0; m<=3; ++m)</pre>
           {
                data1[12+m] = (1 >> m) \& 0x01;
           }
           // Clear the data buffer dataWrite[6] by toggling
           dataWrite[4] 32x
           // corresponding to Dev1/port2/line6 and 4
           respectively
           for (j=0; j<32;++j)</pre>
           {
                dataWrite[6] = 0;
                dataWrite[4] = 0;
                DAQmxErrChk
                (DAQmxWriteDigitalLines(taskHandle,1,1,10.0,DAQ
                mx Val GroupByChannel,dataWrite,NULL,NULL));
                DAQmxErrChk
                (DAQmxReadDigitalLines(taskHandle,1,10.0,DAQmx
                Val GroupByChannel, dataRead, 100, &read, &bytesPer
                Samp,NULL));
```

```
dataWrite[4] = 1;
     DAQmxErrChk
      (DAQmxWriteDigitalLines(taskHandle,1,1,10.0,DAQ
     mx Val GroupByChannel,dataWrite,NULL,NULL));
     DAQmxErrChk
      (DAQmxReadDigitalLines(taskHandle,1,10.0,
      DAQmx Val GroupByChannel, dataRead, 100, & read, & by
      tesPerSamp,NULL));
}
// Uploading data from data1[16] starting with a
leading 1
dataWrite[6] = 1;
dataWrite[4] = 0;
DAQmxErrChk
(DAQmxWriteDigitalLines(taskHandle,1,1,10.0, DAQmx Val
GroupByChannel,dataWrite,NULL,NULL));
DAQmxErrChk
(DAQmxReadDigitalLines(taskHandle,1,10.0,DAQmx Val Gr
oupByChannel,dataRead,100,&read,&bytesPerSamp,NULL));
dataWrite[4] = 1;
DAQmxErrChk
(DAQmxWriteDigitalLines(taskHandle,1,1,10.0,DAQmx Val
GroupByChannel,dataWrite,NULL,NULL));
DAQmxErrChk
(DAQmxReadDigitalLines(taskHandle,1,10.0,DAQmx Val Gr
oupByChannel, dataRead, 100, &read, &bytesPerSamp, NULL);
for (j=0; j<16; ++j)</pre>
{
     dataWrite[6] = data1[j];
     dataWrite[4] = 0;
     DAQmxErrChk
      (DAQmxWriteDigitalLines(taskHandle,1,1,10.0,DAQ
     mx Val GroupByChannel,dataWrite,NULL,NULL));
     DAOmxErrChk
     (DAQmxReadDigitalLines(taskHandle,1,10.0,DAQmx
     Val GroupByChannel, dataRead, 100, & read, & bytesPer
     Samp,NULL));
     dataWrite[4] = 1;
     DAOmxErrChk
     (DAQmxWriteDigitalLines(taskHandle,1,1,10.0,DAQ
     mx Val GroupByChannel,dataWrite,NULL,NULL));
     DAOmxErrChk
      (DAQmxReadDigitalLines(taskHandle,1,10.0,DAQmx
     Val GroupByChannel, dataRead, 100, & read, & bytesPer
     Samp,NULL));
}
dataWrite[4] = 0;
DAQmxErrChk
(DAQmxWriteDigitalLines(taskHandle,1,1,10.0,DAQmx Val
GroupByChannel,dataWrite,NULL,NULL));
```

```
DAQmxErrChk
     (DAQmxReadDigitalLines(taskHandle,1,10.0, DAQmx Val Gr
     oupByChannel,dataRead,100,&read,&bytesPerSamp,NULL));
     if (1<5)
          ao ref value = wash voltage-0.3;
     else if (1 < 12)
          ao ref value = wash voltage;
     else
           ao ref value = wash voltage+0.3;
     ao ref value = wash voltage;
     DAQmxErrChk (DAQmxWriteAnalogScalarF64(taskHandle3,
     0, 10.0, ao ref value, NULL));
     printf("Washing Line: %i Washing Voltage:
     %f\n",l,ao ref value);
     fprintf(LogFile,"Washing Line: %i Washing Voltage:
     %f\n",l,ao ref value);
     start tick=clock();
     while(clock()-start tick<CLK TCK/5)</pre>
     ao ref value=0.0;
     DAQmxErrChk (DAQmxWriteAnalogScalarF64(taskHandle3,
     0, 10.0, ao ref value, NULL));
}
// Wash the last line, address=1111xxx
// Wash cntrl B=000, down=0, up=1, en clk div2=1, Bypass=0,
en0=1, en1=0
for(j=0;j<32;++j)</pre>
{
     dataWrite[6] = 0;
     dataWrite[4] = 0;
     DAOmxErrChk
     (DAQmxWriteDigitalLines(taskHandle,1,1,10.0,DAQmx Val
      GroupByChannel,dataWrite,NULL,NULL));
     DAQmxErrChk
     (DAQmxReadDigitalLines(taskHandle,1,10.0,DAQmx Val Gr
     oupByChannel,dataRead,100,&read,&bytesPerSamp,NULL);
     dataWrite[4] = 1;
     DAQmxErrChk
     (DAQmxWriteDigitalLines(taskHandle,1,1,10.0,DAQmx Val
     GroupByChannel,dataWrite,NULL,NULL));
     DAQmxErrChk
     (DAQmxReadDigitalLines(taskHandle,1,10.0,DAQmx Val Gr
     oupByChannel,dataRead,100,&read,&bytesPerSamp,NULL));
}
// Uploading data from data1[16] starting with a leading 1
```

/*

*/

```
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```

```
dataWrite[6] = 1;
dataWrite[4] = 0;
DAOmxErrChk
(DAQmxWriteDigitalLines(taskHandle,1,1,10.0,DAQmx Val Group
ByChannel,dataWrite,NULL,NULL));
DAQmxErrChk
(DAQmxReadDigitalLines(taskHandle,1,10.0,DAQmx Val GroupByC
hannel,dataRead,100,&read,&bytesPerSamp,NULL));
dataWrite[4] = 1;
DAOmxErrChk
(DAQmxWriteDigitalLines(taskHandle,1,1,10.0,DAQmx Val Group
ByChannel,dataWrite,NULL,NULL));
DAQmxErrChk
(DAQmxReadDigitalLines(taskHandle,1,10.0,DAQmx Val GroupByC
hannel,dataRead,100,&read,&bytesPerSamp,NULL));
for (j=0; j<16; ++j)</pre>
{
      dataWrite[6] = data2[j];
      dataWrite[4] = 0;
      DAQmxErrChk
      (DAQmxWriteDigitalLines(taskHandle,1,1,10.0,DAQmx Val
      GroupByChannel,dataWrite,NULL,NULL));
      DAQmxErrChk
      (DAQmxReadDigitalLines(taskHandle,1,10.0,DAQmx Val Gr
      oupByChannel, dataRead, 100, &read, &bytesPerSamp, NULL));
      dataWrite[4] = 1;
      DAQmxErrChk
      (DAQmxWriteDigitalLines(taskHandle,1,1,10.0,DAQmx Val
      GroupByChannel,dataWrite,NULL,NULL));
      DAQmxErrChk
      (DAQmxReadDigitalLines(taskHandle,1,10.0,DAQmx Val Gr
      oupByChannel,dataRead,100,&read,&bytesPerSamp,NULL));
}
dataWrite[4] = 0;
DAQmxErrChk
(DAQmxWriteDigitalLines(taskHandle,1,1,10.0,DAQmx Val Group
ByChannel,dataWrite,NULL,NULL));
DAOmxErrChk
(DAQmxReadDigitalLines(taskHandle,1,10.0,DAQmx Val GroupByC
hannel,dataRead,100,&read,&bytesPerSamp,NULL));
ao ref value=wash voltage;
DAQmxErrChk (DAQmxWriteAnalogScalarF64(taskHandle3, 0,
10.0, ao ref value, NULL));
printf("Washing Line: 17 Washing Voltage:
%f\n",ao ref value);
fprintf(LogFile, "Washing Line: 17 Washing Voltage:
%f\n",ao ref value);
start tick=clock();
while(clock()-start tick<CLK TCK/5)</pre>
;
ao ref value=0.0;
```

```
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```

```
DAQmxErrChk (DAQmxWriteAnalogScalarF64(taskHandle3, 0,
          10.0, ao ref value, NULL));
     }
     DAQmxStopTask(taskHandle);
     DAQmxClearTask(taskHandle);
     DAQmxStopTask(taskHandle3);
     DAQmxClearTask(taskHandle3);
     DAQmxStopTask(taskHandle5);
     DAQmxClearTask(taskHandle5);
Error:
     if( DAQmxFailed(error) )
          DAQmxGetExtendedErrorInfo(errBuff, 2048);
     if( taskHandle!=0 ) {
     // DAQmx Stop Code
     DAQmxStopTask(taskHandle);
          DAQmxClearTask(taskHandle);
          DAQmxStopTask(taskHandle3);
          DAQmxClearTask(taskHandle3);
     }
     if( DAQmxFailed(error) )
     {
          printf("DAQmx Error: %s\n",errBuff);
          getchar();
     }
     return;
return;
}
```

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