On-chip magnetic separation of superparamagnetic beads for integrated molecular analysis

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We have demonstrated a postprocessed complementary metal oxide semiconductor (CMOS) integrated circuit (IC) capable of on-chip magnetic separation, i.e., removing via magnetic forces the nonspecifically bound magnetic beads from the detection area on the surface of the chip. Initially, 4.5 μm wide superparamagnetic beads sedimenting out of solution due to gravity were attracted to the detection area by a magnetic concentration force generated by flowing current through a conductor embedded in the IC. After sedimentation, the magnetic beads that did not bind strongly to the functionalized surface of the IC through a specific biochemical complex were removed by a magnetic separation force generated by flowing current through another conductor placed laterally to the detection area. As the spherical bead pivoted on the surface of the chip, the lateral magnetic force was further amplified by mechanical leveraging, and 50 mA of current flowing through the separation conductor placed 18 μm away from the bead resulted in 7.5 pN of tensile force on the biomolecular tether immobilizing the bead. This force proved high enough to break nonspecific interactions while leaving specific antibody-antigen bonds intact. A sandwich capture immunoassay on purified human immunoglobulin G showed strong correlation with a control enzyme linked immunosorbent assay and a detection limit of 10 ng/ml or 70 pM. The beads bound to the detection area after on-chip magnetic separation were detected optically. To implement a fully integrated molecular diagnostics platform, the on-chip magnetic separation functionality presented in this work can be readily combine with state-of-the art CMOS-based magnetic bead detection technology.

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I. INTRODUCTION

The past decades have seen the emergence of point-of-care (POC) diagnostics for early disease detection and chronic disease monitoring. The POC glucose monitor is an excellent example of a simple-to-use diagnostic device employed widely. However, introduction and widespread adoption of POC systems for broader molecular diagnostics have been stifled by the difficulty and cost of integrating the functionality of complex protocols involving sample preparation and analyte detection in a low cost, easy-to-use format. Presently, molecular diagnostics are dominated by high throughput laboratory tests such as the enzyme linked immunosorbent assay (ELISA), where enzymatic labels are bound to the surface of polystyrene wells via the target molecule and where excess labels that contribute to the interfering background signal are removed through successive fluidic washing steps. Unfortunately, the ELISA protocol is ill suited for POC application since it requires trained personnel to operate expensive, cumbersome equipment such as a spectrophotometer.

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 POC application: (a) The signals from magnetic beads are not affected by biological interactions and can be detected in opaque solutions such as 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) magnetic beads permit the reduction in incubation times through their high surface area to volume ratio, and through their rapid sedimentation rates that do not rely on slower diffusion processes, and finally (e) the ability to detect individual assay labels obviates the need for lengthy signal amplification.

Integrated circuits (ICs) have been proposed as candidate platforms with which to perform magnetic bead labeled assays.¹ In such assays, specific biomolecular complexes strongly tether magnetic beads to the surface of an IC that contains magnetic sensors. Various on-chip magnetic bead detection systems have been proposed, namely, using Hall sensors,² magnetoresistive spin valves,³ and inductive coils.⁴ However, before the detection of the strongly bound beads can occur, the interfering signal from weakly nonspecifically bound ones must be eliminated. While the detection of the magnetic beads has been addressed at length, the problem of

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FIG. 1. (Color online) On-chip magnetic separation platform. Magnetic beads are pulled to the detection in the center of the trench etched in SiO_2 by magnetic forces generated by current passing through the concentration conductor. Nonspecifically bound beads are pulled aside by magnetic forces resulting from current passing through the separation conductors embedded along the ridges of the trench.

removing the weakly bound magnetic particles remains a key hurdle to implementing effective POC molecular diagnostic platforms.

One possibility consists of using hydrodynamic forces to wash away weakly bound magnetic bead labels.⁵ To do so, the IC must be integrated into a microfluidic 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 nonspecifically bound beads from atop the magnetic sensors. Several groups have used external permanent magnets to generate the magnetic forces that remove the nonspecifically bound beads from the surface of the IC.^{1,6} However, this off-chip magnetic separation requires additional handling steps that can complicate the assay protocol and impact ease of use.

We present an assay cartridge consisting of a gold-coated $0.18\ \mu\text{m}$ complementary metal oxide semiconductor (CMOS) IC capable of generating on chip the magnetic forces that are used to (a) concentrate sedimenting $4.5\ \mu\text{m}$ superparamagnetic beads over the detection area on the surface of the IC, and to (b) remove the nonspecifically bound magnetic beads from the detection area.

CMOS was chosen over competing technologies because of its low cost and design versatility that allows the integration of the magnetic separation, the bead detection, and the back-end circuitry on one single chip. We will show how the high level of integration offered by this platform fundamentally addresses the criteria of what constitutes an effective POC biosensor, namely, high biochemical sensitivity/selectivity, speed, ease of use, and low cost.

The detection range and the sensitivity of a human immunoglobulin G (IgG) immunoassay 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.

II. SYSTEM OVERVIEW

The surface of the CMOS IC capable of autonomously performing on-chip magnetic bead assay label separation is shown in Fig. 1. Magnetic beads sedimenting to the gold-coated surface of the IC are initially drawn by magnetic force

toward the concentration conductor through which current flows. In a process referred to as on-chip magnetic separation, the weakly bound beads that are not strongly tethered by complementary molecular bonds to the surface of the IC are then pulled aside by magnetic forces generated by current passing through separation conductors, placed laterally to the concentration conductor. To overcome the Derjaguin, Landau, Verwey, and Overbeek forces that can lead to inconsistent magnetic separation performance,⁷ the separation conductors are placed along the upper ridges of trenches etched into the SiO_2 , above the plane onto which the magnetic beads settle, thus eliminating the component of the magnetic force that pulls the beads into the surface of the IC. After magnetic separation, the beads remaining strongly bound to the center of the trench are detected optically through a microscope and counted.

III. SYSTEM DESIGN

A. Postprocessing and assembly

The CMOS compatible postprocessing steps for etching the trenches and for depositing the layer of gold are depicted in Fig. 2; the $0.18\ \mu\text{m}$ five metal layer CMOS IC has top metal features to define the trenches. Photoresist is used to protect components such as the connection pads that are also fabricated in the top metal. The IC is then submitted to a reaction ion etch (RIE) to remove all exposed SiO_2 . The top metal features define the trenches while metal 2 features are used as the etch-stop layer at the bottom of the trenches to eliminate the need for timing. Without removing the photoresist, the IC is dipped in an aluminum wet etchant to remove the exposed top metal and metal 2 layers. Last, the photoresist is dissolved and gold is evaporated onto the surface of the trenches through a shadow mask. Figure 3 shows a Scanning Electron Microscope (SEM) of the IC after $4.5\ \mu\text{m}$ M-450 Dynal magnetic beads were applied in solution and dried on the surface.

Four $200\ \mu\text{m}$ long trenches are etched into the surface of the IC, of 20, 24, 28, and $32\ \mu\text{m}$ widths. The different widths were used to optimize the performance of the magnetic separation while minimizing the power consumption.

Figure 4(a) shows a micrograph of the CMOS IC with which the on-chip magnetic concentration and magnetic separation are performed. The gold-coated CMOS ICs are flip-chip bonded to the bottom of printed circuit boards (PCBs) presented in Fig. 4(b). Each PCB houses a $150\ \mu\text{l}$ well with an opening in the center to expose the IC to the solution containing magnetic beads.⁶ In this work, Dynal M-450, $4.5\ \mu\text{m}$ in diameter, superparamagnetic beads are used.⁸ These beads, characterized in Ref. 9, are spherical polymer matrices containing 20% maghemite by weight in the form of 8 nm particles.

B. On-chip magnetic concentration

Figure 5(a) shows a cross section of a trench as a magnetic bead settles 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 toward the center. The detection area

FIG. 2. (Color online) CMOS postprocessing steps. (a) Standard five metal layer $0.18\ \mu\text{m}$ CMOS IC. (b) A RIE step is used to create trenches in the SiO_2 . Photoresist is used to protect the other portions of the IC (connection pads, CMOS circuitry, etc.) while top metal polygons are used to define the trenches. Metal 2 polygons are used as etch-stop layers for the RIE, protecting the concentration conductors buried below the trench. (c) A wet etch is used to remove the exposed aluminum. Note that the connection pads are still protected by the photoresist. (d) The photoresist is removed and 10 nm of Cr followed by 30 nm of Au are evaporated on the sensor area through a shadow mask.

where beads are optically counted is defined as the $10\ \mu\text{m}$ wide strip along the center of the trench. The concentration of magnetic beads to the detection area confers several advantages: (1) Knowledge of where the magnetic beads settle

FIG. 3. SEM of postprocessed IC. Dynal M-450, $4.5\ \mu\text{m}$ wide, superparamagnetic beads are dried on the surface and a SEM is taken of the surface of the IC using a Hitachi TM1000.

FIG. 4. (Color online) CMOS IC and assay cartridge. (a) Micrograph of a $0.18\ \mu\text{m}$ CMOS IC with magnetic bead concentration and separation capabilities. The top metal layer is used as an etch mask to define the trenches in which the assays are performed. (b) Picture of the assembled device, where the IC is flip-chip bonded to the bottom of a PCB housing a $150\ \mu\text{l}$ vial.

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, a fewer total number of beads can be used. (3) On-chip magnetic concentration can be used to pull the magnetic beads directly over magnetic bead sensing elements shown in Ref. 2.

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

FIG. 5. (Color online) Cross section of trench etched in SiO_2 . (a) As the $4.5\ \mu\text{m}$ magnetic beads settle to the surface of the IC due to gravity, current is run through the concentration conductor at the bottom of the trench to ensure that the beads land in the center. (b) After the beads have been concentrated on the surface of the IC, current is passed through the integrated separation conductors running along the ridge of the trench to generate a magnetic force capable of removing all the nonspecifically bound beads, a process referred to as on-chip magnetic separation.

$$a_{\text{sediment}}(t) = \frac{F_{\text{gravity}} - F_{\text{drag}}}{m_b}, \quad (1)$$

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

$$F_{\text{drag}} = 6 \times \pi \times \eta \times r_{\text{bead}} \times v_{\text{sediment}}(t), \quad (2)$$

where the viscosity of water $\eta = 0.89$ g/m s and the radius of the bead $r_{\text{bead}} = 2.25$ μm . By combining Eqs. (1) and (2) and solving for $v_{\text{sediment}}(t)$, we get the following expression:

$$v_{\text{sediment}}(t) = \frac{F_{\text{gravity}}}{6 \times \pi \times \eta \times r_{\text{bead}}} \times (1 - e^{-(6 \times \pi \times \eta \times r_{\text{bead}} / m_{\text{bead}})t}). \quad (3)$$

According to Eq. (3), the 4.5 μm beads settle out of solution to the surface of the IC at a rate of approximately 0.4 mm/min. It is important to note that *ceteris paribus*, 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 sedimentation times are unacceptable.

The magnetic beads approaching the surface of the IC are pulled toward 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 given by¹⁰

$$F_{\text{mag}} = \frac{\mu_o \times \chi_{\text{bead}} \times r_{\text{bead}}^3 \times I_{\text{sep}}^2}{3 \pi \times x_{\text{bead}}^3}. \quad (4)$$

The concentration conductors are implemented in metal 1 and placed 0.75 μm underneath the detection area in the center of the trenches. 2 mA flowing through these 2 μm wide conductors pulls the magnetic beads toward the detection area with a force of approximately 0.2 pN from a distance of $x_{\text{bead}} = 4$ μm [Fig. 5(a)].

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 $x_{\text{bead}} > 100$ μm . 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.

C. Magnetic separation

After concentration, the nonspecifically 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 Fig. 5(b). 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.

To perform an immunoassay, the magnetic forces must be sufficiently strong to pull the nonimmunologically bound beads to the side of the trench (> 0.1 –10 pN),⁵ away from the detection area, but not overly strong so as to remove the immunologically bound ones (< 60 pN).¹¹ According to Eq.

FIG. 6. (Color online) Mechanical leverage. The magnetic force F_{mag} applied to the lever arm of height r_{bead} translates into a magnified force F_{tether} on the last immunological tether of length L (Ref. 5).

(4), 50 mA of current flowing through the separation conductors pulls a magnetic bead resting in the center of the trench with 1.1 pN 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 force⁵ (Fig. 6). The expression of F_{tether} , the force on the last molecular tether of length L , is given by

$$F_{\text{tether}} = F_{\text{mag}} \sqrt{\frac{r_{\text{bead}}}{2L}}. \quad (5)$$

In this case, the longest possible nonspecific tether consists of three cross-reactive antibodies, which total 25.5 nm in length.¹² The 1.1 pN lateral magnetic force translates into a tensile 7.5 pN force on the last tether, sufficient to remove nonspecifically bound beads.

Equation (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 on chip to permit digital modulation of the currents.

D. 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 50 mA through the 2 μm wide, 0.5 μm high, and 200 μm long conductors must be minimized. The heat generated is dissipated in several ways, the two most important being transferred through the IC and storage in the thermal capacitance of the fluid in the well. Figure 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 as

$$\Delta T = (T_{\text{final}} - T_{\text{ambient}})(1 - e^{-t/\tau}), \quad (6)$$

where the steady-state final temperature $T_{\text{final}} = P_{\text{in}} \times (R_{\text{th2,SiO}_2} + R_{\text{th,Si}})$ and the thermal time constant $\tau = (R_{\text{th1,SiO}_2} + R_{\text{th2,SiO}_2} + R_{\text{th,Si}}) \times C_{\text{th,fluid}}$ 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 $P_{\text{in}} = 20$ mW for 30 s

FIG. 7. (Color online) Lumped equivalent thermal circuit. The two forms of heat dissipation modeled are transfer through the backside of the IC and storage in the thermal capacitance of the fluid inside the well. A magnetic separation power $P_{in}=20$ mW applied for 30 s will increase the temperature of the fluid in the well by 2.2 °C above ambient temperature.

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.

E. Selection of magnetic beads

The 4.5 μm wide Dynal M-450 magnetic beads used for this work are larger than those used in past magnetic bead assays performed on chip.⁸ The use of larger magnetic beads as assay labels has the following disadvantages.

- (1) The larger contact area between the beads to the surface of the IC leads to more nonspecific interactions and therefore to higher nonspecific binding forces. This is mitigated through surface biochemical 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.

Most importantly, however, larger forces can be exerted on the 4.5 μm wide beads 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 4.5 μm wide magnetic beads are selected.

F. Surface biofunctionalization

The biochemical sensitivity of the on-chip protocol is determined by assaying known concentration of purified human IgG.⁶ Figure 8 depicts the nature of the immunological sandwich capture complex immobilizing the beads to the detection area. The gold surface is first coated with surface antibodies, polyclonal goat IgG specific to the F_{ab} region of human IgG (Sigma Aldrich). The surface is then biochemi-

FIG. 8. (Color online) Magnetic bead binding chemistry. Surface polyclonal goat IgG specific to the F_{ab} region of human IgG is passively adsorbed on the gold surface. Human IgG antigen is added, followed by the primary biotinylated monoclonal goat IgG specific to the F_c region of the human IgG antigen. Last, the streptavidin-coated 4.5 μm Dynal bead labels are added.

cally passivated with nonfat dried milk (NFDM) before the human IgG antigen is introduced (Sigma Aldrich), followed by the primary antibody, a biotinylated monoclonal goat IgG specific to the F_c region of human IgG (Sigma Aldrich). Finally, the streptavidin-coated 4.5 μm Dynal bead labels are added in a solution of Phosphate Buffer Solution (PBS) with NFDM and bovine serum albumin (BSA) (Sigma Aldrich).

G. Control assays

The on-chip assay conjugation immunochemistry 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 covered silicon slides are used to determine the performance of assays run on gold surfaces versus assays run in polystyrene wells.
- (3) An off-chip 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 2.8 μm wide magnetic beads were used rather than Dynal M-450 4.5 μm wide beads, since the latter aggregate when magnetically separated using a permanent magnet due to the strong fringe magnetic fields.

IV. METHODS

A. CMOS postprocessing and assembly

Single, diced ICs were first mounted onto a 4 in. holding wafer using a drop of photoresist applied manually with a pen (Printed Circuit Marker, IKW Mark-Tex). The photoresist was hard baked for 1 h 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 h at 120 °C. The wafer was placed in the RIE chamber (auto-etch plasma etch system, Lam Research) to remove the exposed SiO₂ (Fig. 4). The plasma was fired for 20 s at a time, 12 times, with 90 s intervals in between to allow the sample to cool. The wafer with the mounted ICs was then dipped in an aluminum etch for 2 min (80% H₃PO₄, 5% HNO₃, 5% CH₃COOH, and 10% DI, Transene Co., 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 deionized (DI) water and dried. The ICs were then placed upside down in a 4 in. 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 and EB3 multihead electron beam source, BOC Edwards), which was pumped down to 10⁻⁵ Torr. Then 10 nm of Cr followed by 30 nm of Au were deposited through the shadow mask.

After postprocessing, the ICs are flip-chip bonded to the bottom of a PCB. The top of the PCB houses a 150 μ l 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 Corwil Technologies (Fig. 2).

B. 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 100 μ l of antihuman Fab-specific IgG manufactured in goat (Sigma Aldrich) for 3 h at room temperature, at a concentration of 5.3 μ g/ml. The solution was then discarded, and the wells blocked with 120 μ l of 0.3% NFDM for 16 h at 4 °C. Wells were then washed three times in 200 μ l of 0.05% PBS-Tween, and cleaned wells were incubated with 100 μ l of purified human IgG (Sigma Aldrich) for 1 h at room temperature. Tenfold dilutions of purified human IgG were tested ranging from 1 mg/ml down to 100 pg/ml along with a no-IgG control. After incubation with human IgG wells were washed four times in PBS-Tween, then incubated with 100 μ l of 500 ng/ml biotinylated anti-human Fc-specific IgG manufactured in goat (Sigma Aldrich) for 1 h at room temperature. This was followed by another four time wash in PBS-Tween. Wells were then incubated with 100 μ l of 2.8 μ g/ml streptavidin-linked alkaline phosphatase (Pierce Biotech) diluted in PBS-Tween for 1 h. After washing the wells five times with PBS-Tween, 100 μ l of paranitrophenyl phosphate (pNPP) (Pierce Biotech) were added to each well in quick succession, and the catalytic reaction was allowed to progress for 1 h. At this point the reaction was halted with 100 μ l of 3M NaOH, and the plates were read on a BioTek EL808 ELISA reader at a wavelength of 405 nm.

C. 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 multiarray slide module (Grace Bio-Laboratories). Wells were filled with 130 μ l of antihuman Fab-specific IgG manufactured in goat (Sigma Aldrich) for 3 h at RT at a concentration of 5.3 μ g/ml, then blocked with 150 μ l of 0.3% NFDM for 16 h at 4 °C. The wells were then washed three times in PBS-Tween and incubated with 130 μ l of purified human IgG (Sigma Aldrich). Eight wells were filled with each Human IgG concentration, ranging from 1 μ g/ml to 100 pg/ml with a dilution factor of 1/10 for 5 aliquots total. After washing four times in PBS-Tween, the wells were incubated with 100 μ l of 500 ng/ml biotinylated antihuman Fc-specific IgG manufactured in goat (Sigma Aldrich) for 1 h at RT. A four time PBS-Tween wash prepared the samples for either a gold surface ELISA or a bead-count assay.

D. Gold surface based ELISA labeling and detection

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

E. Magnetic bead based control assay labeling and detection

Streptavidin-coated paramagnetic 2.8 μ m (M-280 Dynabeads, Invitrogen) were blocked at a 1:1:1:7 proportion of beads:1.5% NFDM:1.5% BSA:PBS for 3 h 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 μ l of this solution was pipetted into the wells in sets of eight, and the beads were allowed to settle for 10 min before they were washed with a row of 6/16 in. neodymium rare-earth block magnets (K&J Magnetics) attached side to side, with magnetic poles directed up and down. The row of magnets was gently slid back and forth over the tops of the wells three times, then allowed to rest for 2 min 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 three nonoverlapping pictures corresponding to 1 mm² were taken in the center of each well at 200 $\times$ magnification using a Charge Coupled Device (CCD) camera (Micromanipulator Co. Inc., Moticam Inc.). The beads present in the three pictures from each well were counted and added together automatically using a MATLAB application.

FIG. 9. (Color online) Control assays results. Serial tenfold dilutions of purified human IgG from 1 $\mu\text{g/ml}$ to 100 pg/ml were assayed to compare the biochemical sensitivity and dynamic range of ELISA on polystyrene, ELISA on gold, and off-chip magnetic bead labeled assays. The experiment at each dilution was repeated eight times and the error bars correspond to ± 1 SD.

It is important to note that the beads used in this control assay were 2.8 μm Dynal M-280 rather than the 4.5 μm Dynal M-450 beads used for the on-chip assay. The 4.5 μm 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 2.8 μm beads give a reasonable indication of the performance of bead based assay labeling.

F. On-chip purified human IgG assay

Streptavidin-coated 4.5 μm (M-450 Dynabeads, Invitrogen) were blocked in a 1:1:8 solution of beads:5% NFD-M:PBS for 3 h on slow rotation. The PCB wells were incubated with 100 μl of antihuman Fab-specific IgG manufactured in goat (Sigma Aldrich) for 3 h at room temperature, at a concentration of 5.3 $\mu\text{g/ml}$. The solution was then discarded, and the wells were blocked with 120 μl of 0.3% NFD-M for 16 h at 4 $^{\circ}\text{C}$ and then washed three times with 200 μl of 0.05% PBS-Tween. Experiments with different aliquots of human IgG 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, 100 μl of 0 ng/ml of human IgG was introduced into the well and incubated for 1 h at room temperature. After incubation, wells were washed four times in PBS-Tween, then incubated with 100 μl of 500 ng/ml biotinylated antihuman Fc-specific IgG manufactured in goat (Sigma Aldrich) for 1 h at room temperature. This was followed by another four time washing step in PBS-Tween. 40 μl of a 1/50 dilution of blocked streptavidin 4.5 μm beads were added to the wells and let settle for 2.5 min with 2 mA of current flowing through the concentration line. Afterwards, the magnetic washing conductors on alternating sides of each trench were pulsed with 50 mA of current at 0.3

Hz for 30 s. 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 three times 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 1 to 100 ng/ml in tenfold 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.

V. RESULTS

A. Control assays

Standard curves for serial tenfold dilutions of purified human IgG from 1 $\mu\text{g/ml}$ to 100 pg/ml were produced using conventional ELISA on polystyrene, ELISA on gold, and the off-chip magnetic bead labeled assay. Figure 9 shows the comparison of the biochemical sensitivity and the dynamic range of the three protocols. The conventional ELISA had a sensitivity limit of 10 ng/ml, with an upper detection range at 100 ng/ml. ELISA on gold performed better; concentrations down to 1 ng/ml were detected reliably with an upper detection range of 100 ng/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 1 ng/ml and an upper detection maximal at 100 ng/ml.

Of importance, the ratio of the 100 ng/ml signal to the negative control for the magnetic bead based assays was over twice higher than the ELISAs, and the linear dynamic range

FIG. 10. (Color online) Magnetic bead concentration. The time lapse micrograph shows the $4.5\ \mu\text{m}$ beads settling onto the surface of the IC as 2 mA of current are run through the concentration conductors under the center of the $28\ \mu\text{m}$ wide trench. The shaded rectangle delineates the $10\ \mu\text{m}$ wide detection area in which the magnetic beads are counted.

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 h versus 16 h), the sensitivities of the control assays presented in this work are below those presented in Ref. 6. These results are nonetheless consistent with rapid, compact, commercially available ELISA protocols.¹³

B. Magnetic bead sedimentation and concentration

The measured settling rates for the M-450 magnetic beads and the M-280 beads settled were 0.32 and 0.07 mm/min, respectively. Figure 10 shows the time lapse micrographs of the $28 \times 200\ \mu\text{m}^2$ trench, taken over 2.5 min, as M-450 beads sedimented to its surface. The concentration conductors had 2 mA of current flowing through them to pull the beads to the $10\ \mu\text{m}$ wide detection area delineated by the shaded rectangle. In four 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 four experiments where 2 mA of current flowed through the magnetic concentration conductor, the proportion of beads landing in the detection area increased to 55%. Higher magnetic concentration forces resulted in even more efficient magnetic bead concentration, but the use of currents in excess of 2 mA led to stronger nonspecific interaction between the beads and the surface of the trenches, leading to a loss in assay sensitivity.

C. On-chip magnetic bead separation

The on-chip magnetic separation was started immediately after the 2.5 min needed for the magnetic beads to settle. The few beads that had not settled prior to the magnetic separation were simply pulled toward the magnetic separation conductors, away from the detection area in the center of the trench. Figure 11 shows the micrographs of a

FIG. 11. (Color online) On-chip magnetic bead separation. Micrographs of a trench before (left) and after (right) magnetic separation are shown for 0, 1, and 10 ng/ml concentrations of human IgG antigen. The separation current $I_{\text{sep}} = 50\ \text{mA}$ is alternated between the left and right sides of the trench at frequency $f_{\text{sep}} = 0.3\ \text{Hz}$ for $t_{\text{sep}} = 30\ \text{s}$.

trench before (left) and after magnetic separation (right) for 0, 1, and 10 ng/ml concentrations of purified human IgG antigen.

A separation current of $I_{\text{sep}} = 50\ \text{mA}$ for a duration of $t_{\text{sep}} = 30\ \text{s}$ was needed to remove all the nonspecifically 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 $f_{\text{sep}} = 0.3\ \text{Hz}$. Force modulation frequencies over 1 Hz 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 0.1 Hz 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 $I_{\text{sep}} = 50\ \text{mA}$ the separation time was limited to 30 s after which time no bead displacement was observed.

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

D. On-chip human IgG assay results

The standard curve for tenfold dilutions of purified human IgG from 100 to 1 ng/ml and one negative control was produced using on-chip magnetic separation. The results from two independent experiments are presented in Fig. 12. The 1 ng/ml data points are higher than their corresponding negatives for both on-chip assays, but taken in aggregate and in absolute terms, human IgG was detectable at a concentration of 10 ng/ml, or 70 pM, with a correlation factor of 0.995 to the conventional ELISA controls shown in Fig. 9. While the on-chip assay sensitivity is in line with the conventional ELISAs performed in this work, it is important to note that a much smaller surface area was used: one $28 \times 200\ \mu\text{m}^2$ trench for the on-chip protocol as opposed to $97\ \text{mm}^2$ for the ELISA.

FIG. 12. (Color online) On-chip assay results. The number of beads remaining in the detection area after magnetic separation for serial tenfold dilution of purified human IgG is presented.

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 nonspecifically bound ones. Moreover, the CMOS-based magnetic separation required no user intervention, making it an ideal technology for POC molecular diagnostic tools.

VI. DISCUSSION AND FUTURE WORK

The most important characteristics of an effective POC molecular diagnostic tool are high biochemical sensitivity and selectivity, fast operation, low cost, and ease of use. The manners in which these issues are fundamentally addressed with on-chip magnetic separation are discussed below.

Sensitivity. The high biochemical 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 immunological interactions rather than aggregates statistically reduces background noise.

Selectivity. The fine calibration of the magnetic forces applied to the molecular tethers greatly benefits biochemical selectivity by enabling the precise removal of magnetic beads that are cross-reactively bound to the surface of the chip.

Speed. The use of on-chip magnetic separation speeds up the assay label incubation and separation steps (3 min in total). In addition, signal amplification is not necessary in a system capable of detecting individual magnetic beads.

Ease of use. Ease of use can be achieved by integrating as much as possible the assay protocol on chip. We have previously demonstrated on-chip detection,² which can be combined with the on-chip magnetic separation in a configuration shown in Fig. 13. In such an implementation, magnetic beads are pulled atop Hall sensors by magnetic forces generated by current passing through the microcoils embedded underneath the center of the trench. Nonspecifically bound beads are pulled aside by magnetic forces resulting from current passing through the separation conductors. Last, the specifically bound beads are magnetized by the microcoil

FIG. 13. (Color online) Fully integrated assay platform. Magnetic beads are pulled atop Hall sensors by magnetic forces generated by current passing through the microcoils embedded underneath the center of the trench etched in SiO₂. Nonspecifically bound beads are pulled aside by magnetic forces resulting from current passing through the separation conductors embedded along the ridges of the trench. Last, the specifically bound beads are magnetized by the microcoil and detected by Hall sensors also embedded below the center of the trench (Ref. 2).

and detected by Hall sensors stacked underneath the microcoils.²

Cost. The use of CMOS greatly reduces the overall system costs by allowing the integration of various assay components (label manipulation and detection) alongside the circuitry necessary for extracting and digitizing the results. In terms of production costs, nonrecurring development costs can be contained by employing legacy technology nodes with standard design flows, while variable costs are low due to the batch manufacturing and high scales achievable with relative ease. This cost advantage can be translated into performance by increasing the effective assay surface area or into added functionality by adding temperature sensors and/or wireless capabilities.¹⁴

In addition to these criteria, 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 nonspecific binding characteristics at the surface of the IC.⁷ As an extension to this concept, large control forces can be applied to ensure the appropriate affinity of the specific complementary bonds and therefore the validity of the assay. Last, comprehensive on-chip controls and detection of panels of biomarkers are possible with multiplexed operation.¹⁵

While the initial results are encouraging, statistical significance and repeatability work were restricted due to the limited number of chips available for this work. Moreover, the surface functionalization can be improved through the use of heterobifunctional cross-linkers that bind and orient the antibodies densely on the gold surface.¹⁶ Last, an array of 28 μm wide trenches can be used to increasing the effective assay area while the use of smaller beads may reduce the nonspecific interactions at the surface.

VII. CONCLUSION

We have successfully demonstrated on-chip magnetic separation of 4.5 μm 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 10 μm wide detection area in the center of a 28 μm wide $\times$ 200 μm long trench etched in the SiO_2 . The proportion of beads landing in the detection area rose from 36% without magnetic concentration to 55% with magnetic concentration. After sedimentation, the nonspecifically bound beads were removed from the detection area by a magnetic force generated by passing 50 mA of current through conductors embedded along the ridges of the trench, 18 μm 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 0.3 Hz for 30 s. Due to mechanical leveraging, a 1.1 pN lateral magnetic force translated into tensile 7.5 pN on the biochemical tether immobilizing the bead. This on-chip magnetic separation functionality was applied to an immunoassay on purified human IgG samples and concentrations down to 10 ng/ml or 70 pM 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, integration with Hall sensors for the detection of magnetic beads can lead to a fully integrated molecular diagnostic platform. Such high levels of integration are necessary for enabling effective POC diagnostic capabilities and low cost proteomic and genomic research tools.

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