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A microfluidic sensor based on ferromagnetic resonance induced in magnetic bead labels

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Abstract: This report details preliminary studies towards the development of a microfluidic sensor that exploits ferromagnetic resonance, excited in magnetic bead labels, for signal transduction. The device consists of a microwave circuit in which a slotline and a coplanar waveguide are integrated with a biochemically activated sensor area. The magnetic beads are immobilized in the sensor area by bio-specific reactions. A microwave signal applied to the slotline is coupled to the coplanar waveguide only in the presence of magnetic beads at the functionalized sensor area. Ferromagnetic resonance in the beads further enhances the coupling. This inductive detection technique lends itself to miniaturization, is inexpensive to fabricate and can be adapted for the detection of a wide range of molecules for which bio-specific ligands are available.

Experimentally, the variation of the output signal as a function of the location of magnetic beads was studied for the proposed technique. Subsequently, a prototype device was constructed by biotinylation of the sensor area and integration with a microfluidic chip fabricated in polydimethyl siloxane (PDMS). Preliminary experiments were conducted on this prototype using streptavidin-functionalized magnetic beads as labels. It was shown that the magnetic beads, immobilized at the sensor area by streptavidin-biotin linkage, produced a distinct ferromagnetic resonance response easily discernable from the background signal.

Keywords: Microfluidics, Sensors, Ferromagnetic resonance, Magnetic beads

1. Introduction

An important step towards the development of a portable, standalone, point-of-use diagnostic device involves integration of a highly sensitive transduction system with the sensing module. Typically, immunoassay-based techniques employ a label attached to a biochemical probe for detection. Labels may be molecules such as radioisotopes, fluorescent dyes or enzymes, or may be particulate in nature, such as quantum dots and nanoparticles. Label-free methods of detection, such as surface plasmon resonance (SPR) and quartz crystal microgravimetry (QCM) are gaining popularity as they bypass the traditional labeling steps to provide a rapid and highly specific means of identifying and quantifying analytes. While these label-free techniques offer advantages in terms of reduced assay time, which is essential in the field of rapid-onset-illness diagnostics, they cannot at present match the detection limits of traditional labeled assays such as ELISA or radioimmunoassay (RIA) [1]. SPR can only detect molecules in close proximity of the sensing surface, which is not suitable for typical microfluidic devices since they have channel heights much greater than 200 nm [2]. Also, instrumentation based on QCM and SPR are not as amenable to miniaturization and batch fabrication [3]. Conventional techniques for detection of labeled molecules are highly sensitive but suffer from certain drawbacks. Radioactive labels have a limited shelf life and stringent waste disposal requirements. Optical detection requires the use of instrumentation that is bulky and difficult to miniaturize [4]. Thus, there is a need for investigation into alternative methods of transduction that are highly sensitive, conducive to miniaturization and suitable for mass production.

This report presents a novel high-sensitivity inductive detection technique for potential immunosensing applications using magnetic beads as labels: a microwave circuit, designed for operation at frequencies from 2 GHz to 4 GHz, was used to excite ferromagnetic resonance (FMR) in beads immobilized at the sensor area and detect the resulting signal. The biotin-avidin pair was employed to demonstrate the potential of this technique for bio-specific detection. High sensitivity was achieved both by operating at high frequency and matching the microwave excitation to the ferromagnetic resonance frequency in the beads. To our knowledge, this is the first report that demonstrates inductive detection of FMR excited in magnetic bead labels for biosensing applications. In contrast to commonly proposed magnetoimpedance [5-11] or Hall-effect [12-13] sensors, the microwave circuit requires no specialty thin films or complex processing. The circuit was implemented in a single metal layer and fabricated using standard, inexpensive, integrated circuit (IC) processes. This report establishes the feasibility of using FMR-based detection of magnetic labels towards an inexpensive, mass-producible, hand-held sensor for biomolecules. In the following sections, the principle of operation, fabrication techniques, experimental setup and results are elaborated.

2. Description of Sensor Operation

[Insert figure 1 here]

As shown in Fig.1, the microwave circuit consists of a slotline and a coplanar waveguide (CPW). The junction between the slotline and coplanar waveguide is the active sensor area, which may be functionalized with analyte-specific ligands such as antibodies or aptamers. In this study, the avidin-biotin pair was employed to demonstrate biospecific interactions due to unique properties such as (i) the exceptionally strong interaction between biotin and avidin ($K_a = 10^{15} \text{ M}^{-1}$) (ii) availability of multiple sites on avidin for binding biotin, so that it can act as a cross-linker between two biotinylated moieties (iii) easy commercial availability of a wide variety of biotinylated antibodies and aptamers, which allows incorporation of these bio-specific ligands in the device in future. The waveguides were patterned in thin-film aluminum, thermally evaporated on a glass substrate. The active sensor area was defined using a facile method of photo-activated patterning of biotin.

When the slotline is excited by a microwave signal, *ac* magnetic fields are generated at the junction. These fields are orthogonal to the electromagnetic wave modes allowed in the coplanar waveguide. Consequently, no signal couples from the slotline into the coplanar waveguide. When magnetically labeled moieties are immobilized at the junction, the field distribution is perturbed resulting in the input signal being inductively coupled to the output at the coplanar waveguide. The output signal is proportional to the frequency of microwave excitation and is further enhanced by stimulating ferromagnetic resonance in the beads. The design of the microwave waveguides has previously been reported in [14] and is based on equations found in [15-16]. The condition for FMR is described as follows [17]:

$$\omega_{FMR} = \gamma \mu_0 H_{DC} \quad (\text{Eq. 1})$$

where ω_{FMR} is the FMR frequency; γ , the gyromagnetic ratio ($\sim 175 \text{ GHz/Tesla}$); μ_0 , the permeability of free space and H_{DC} , a *dc* magnetic field bias. The bias field must be adequate to saturate the magnetic beads to ensure that their magnetization precesses in phase at resonance.

3. Materials and methods

3.1. Materials and reagents

Superparamagnetic beads, 1 μm in diameter, were obtained from MagSense Life Sciences (West Lafayette, IN, USA). Streptavidin coated magnetic beads (nanomag-D, 250 nm) were purchased from Micromod (Rostock-Warnemuende, Germany). Bovine serum albumin (BSA), (3-aminopropyl) triethoxysilane (APTS) and dimethyl sulfoxide, anhydrous (DMSO) were obtained from Sigma Aldrich (St. Louis, MO, USA). A photoactivable form of biotin with tetrafluorophenyl azide moiety, EZ-Link TFPA-PEG₃-Biotin, and fluorescein labeled Neutravidin were purchased from Pierce Biotechnology, Inc. (Rockford, IL, USA). SU-8 3050, a

negative tone, epoxy based Photoresist was purchased from Microchem (Newton, MA, USA). Sylgard® 184 (Silicone elastomer kit) comprised of polydimethyl siloxane (PDMS) prepolymer and curing agent was obtained from Dow Corning Corp. (Midland, MI, USA). Corning 1737 (Corning, NY, USA) one inch square glass slides were used as the substrate for the detection circuit.

All general reagents and solvents were procured from Sigma Aldrich or VWR (West Chester, PA, USA). The phosphate buffer solution (PBS) consisted of 10 mM phosphate-buffered saline and 149 mM NaCl (pH 7.4). The blocking buffer used was a 1% (w/v) solution of BSA in PBS (pH 7.4). All chemicals were used as received, without further purification. Ultrapure water, with specific resistance of 18 M Ω -cm, was obtained from a Barnstead™ E-Pure™ deionization system (Waltham, MA) and was used for rinses and the preparation of buffers throughout the experiments. An Axiotron Inspection Microscope (Carl Zeiss MicroImaging, Germany) with an attached camera (Model TCA-3.0C from Tucsen Imaging Technology, Fujian, China) was used for acquiring the images shown in Fig. 7.

3.2. Microwave circuit fabrication

The microwave circuit was fabricated using photolithography and wet chemical etching. A 500 nm film of aluminum was deposited onto a one inch square glass slide by thermal evaporation. The waveguides were then patterned using a 1:1 chrome mask and Karl Suss MJB3 (Garching, Germany) contact aligner to expose positive photoresist (Shipley 1818, Microchem) spin coated onto the slide. The exposed aluminum was etched in a standard aluminum etchant (16:2:2:1 solution of H₃PO₄, H₂O, HNO₃, CH₃COOH) to form the waveguides. The remaining photoresist was removed with acetone. Finally, a 500 nm layer of silicon dioxide was sputtered onto the sensor surface for insulation and subsequent functionalization with biotin (as detailed in section 3.5.1.).

The waveguides were designed to allow propagation of frequencies in the 2-4 GHz range. As determined by Eq. 1, these frequencies correspond to the *dc* bias field required to saturate the magnetic beads. The microwave circuit was modeled for Corning 1737 glass substrate. Details of the modeling parameters used and the results obtained were published in an earlier report [14]. Briefly, both the slotline and CPW were designed to have a 50 Ω characteristic impedance to match available instrumentation (section 3.3. and depicted in Fig.2). The slot line is 78 μ m wide and the CPW has a 78 μ m center conductor separated from the ground plane on both sides by 28 μ m gaps. The distance from the end of the slotline to the CPW is 50 μ m providing an active sensor area of approximately 50 μ m by 78 μ m (Fig. 5a). In order to allow the slotline to be driven from a coaxial cable, a $\frac{1}{4}$ -wave stub, CPW-to-slotline transformer was also patterned on the glass slide. External electrical connections were made to the waveguides by wire bonding to a printed circuit board having coaxial cable connectors.

3.3. Electronic Instrumentation

[Insert figure 2 here]

The laboratory instrumentation used to measure the magnetic bead response of the sensor is shown in Fig. 2. A network analyzer (ENA5071C, Agilent Technologies, Santa Clara, CA) was used to excite the slotline with a microwave signal and monitor the signal coupled to the CPW. The excitation frequency, chosen to be 2.7 GHz in these experiments, lies within the designed range of operating frequencies for the microwave circuit. The network analyzer measures the transmission parameter, S_{21} - i.e. the ratio of microwave power received at the CPW to the power input at the slotline. The detected S_{21} signal depends on the number and location of magnetic beads present at the junction area and peaks when the excitation frequency matches the ferromagnetic resonance frequency set by the *dc* bias field (Eq. 1).

The bias field was applied using a C-shaped iron core wound with two independent wire coils. The first winding is used to generate a *dc* field using a programmable power supply (Model 2400, Keithley Instruments, Cleveland, OH). The microwave circuit was placed within the gap of the C-core so that the magnetic fields were perpendicular to the plane of the device. The second winding on the C-core was used to modulate the *dc* field at 160 Hz to allow lock-in detection of the signal. Since the FMR response is a strongly non-linear function of the bias field, the modulation results in a second harmonic (320 Hz) variation in the amplitude of the microwave signal coupled to the CPW. Detection of this second harmonic component uniquely identifies the FMR effect of the magnetic beads and distinguishes the signal from stray, field-independent coupling as well as possible feed-through of the modulation signal. The current in the modulation coil, supplied by an arbitrary waveform generator (N8242A, Agilent Technologies, Santa Clara, CA) and audio amplifier, provided an *ac* field amplitude of 42 mT.

The S_{21} signal, at 2.7 GHz, was measured as the *dc* bias field was varied from 0 to 0.2 T. The second harmonic (320 Hz) modulation in the 2.7 GHz carrier was then extracted from the S_{21} data using custom software implemented in MATLAB and plotted as a function of the *dc* bias field (Fig. 6c).

3.4. Characterization of variation in sensitivity

The spatial variation in sensitivity was characterized by measuring the output signal from a fixed number of beads placed at varying locations on the sensor area. Approximately 40 MagSense beads, glued to the end of a fine-tipped glass probe, were rastered across the active sensor area using an x-y stage. The signal obtained at each location was then mapped as shown in Fig. 4.

3.5. Sensor area functionalization and characterization

3.5.1. Photo-activated patterning of biotin on sensor area

[Insert figure 3 here]

The active sensor area (the area between the slotline and CPW where detection will occur) is approximately 50 μm by 78 μm . However, a much larger area (about 500 μm by 1000 μm) was deliberately chosen for biotin patterning. This was done in order to facilitate easy alignment of the sensor area with the pattern for UV exposure, within the limitations imposed by our exposure system. The waveguide substrate was passivated with silicon dioxide prior to biotin patterning both for electrical isolation and to provide a compatible layer for functionalization. The reaction scheme for the functionalization of the sensor area is depicted in Fig. 3. The silica-coated device was rinsed with deionized water followed by isopropanol. The sensor area between the slotline and co-planar waveguides was treated with 50 μL of 2% (v/v) aqueous solution of APTS for 1 hour. The device was rinsed with deionized water and dried overnight in an oven at 100°C. 20 μL of a 10 mg/mL solution of EZ-Link TFPA-PEG₃-Biotin in DMSO was deposited on the sensor area and allowed to dry at 40°C for about 2 hours. A maskless exposure system, SF-100 (Intelligent Micro Patterning, LLC, St. Petersburg, FL), was used to align and pattern a 500 μm by 1000 μm area encompassing the sensor using UV light. An exposure time of 30 minutes was used. Immediately after UV exposure, the slide was rinsed with PBS to wash away any unconjugated biotin. The sensor area was incubated with 100 μL of the blocking buffer for 15 minutes followed by rinsing with a copious amount of PBS.

3.5.2. Verification of biotin patterning by fluorescence microscopy

For verification of the biotin patterning by fluorescence microscopy, the sensor area was treated with 200 μL of a 2 mg/mL solution of Neutravidin-fluorescein in PBS for 30 minutes. The devices were rinsed with PBS prior to imaging by an inverted fluorescence microscope, Olympus IX-71 (Olympus, Center Valley, PA) equipped with a PIXIS-512 cooled CCD camera (Princeton Instruments, Trenton, NJ). The camera was controlled by WinSpec32 software (Princeton Instruments, Trenton, NJ). A 300 Watt xenon arc lamp was employed as the light source. The setup used for imaging was: a 40x/0.17 objective (UplanSApo, Olympus, Center Valley, PA), a 450/50 nm excitation filter and a 515 nm long pass emission filter.

3.6. Fabrication of microfluidic chip

The microfluidic chips used in this study were fabricated by replica molding of PDMS from a SU-8 master. The SU-8 master, containing negative-relief of the microchannel, was fabricated employing standard photolithographic techniques as described in an earlier study [18]. To obtain a PDMS replica, a 10:1 (w/w) mixture of the prepolymer and curing agent was mixed thoroughly and allowed to degas under vacuum. The SU-8 master was placed in a mold and the degassed prepolymer-curing agent mixture was poured on it. The assembly was then placed overnight in an oven at 65 °C to cure the polymer. Subsequently, the assembly was removed from the oven and allowed to cool to room temperature, prior to gently peeling off the PDMS replica from the SU-8 master. The chip thus obtained was a single, straight flow-through channel ~ 100 μm wide,

~ 100 μm deep and ~ 2 cm in length. Inlet and outlet holes were punched into the PDMS chip using a belt-puncher.

3. 7. *Demonstration of detection technique*

The detection technique was demonstrated by measuring the output signal obtained from beads immobilized at the sensor area due to biotin-avidin binding. The microchannel fabricated earlier in PDMS was carefully aligned, under an optical microscope, to the biotin-patterned sensor area. In all subsequent steps, fluid was introduced into the microchip using a syringe and drawn out using tubing connected to house-vacuum. Prior to experiments, the PDMS chip was conditioned with a solution of 1% BSA (w/v) in PBS for 30 minutes to minimize non-specific binding of magnetic labels. The chip was thoroughly rinsed by flowing copious amounts of PBS. Subsequently, 10 μL of a 1mg/mL suspension of nanomag-D streptavidin-coated magnetic beads was introduced into the chip. A magnetic field gradient, to attract the beads to the junction area, was created by applying a *dc* current (160 mA) at the terminals of the CPW. After 15 minutes, the current was switched off and the unbound beads removed by rinsing with PBS. The chip with the immobilized beads was placed in the gap of the C-core and the FMR signal measured. For a control, the experiment was also repeated on a second device without biotin patterning.

4. Results and discussion

4.1. *Characterization of spatial variation in sensitivity*

[Insert figure 4 here]

MagSense beads affixed to a glass probe were scanned over the active sensor area. The output signal varied with the location of the beads, as illustrated by the color map in Fig. 4. The highest sensitivity, shown in dark red, was observed around the inner tips of the CPW. The spatial variation in sensitivity arises due to non-uniformity in the microwave magnetic field within the active sensor area. The distribution of the field depends on the geometry and layout of the waveguides, and was discussed in [14]. Intuitively, a larger output signal can be expected if the beads are immobilized closer to the pickup waveguide (CPW). These measurements enable placement of the fluidic channel and the bio-chemically activated area to coincide with regions of highest detection sensitivity.

Additionally, it should be noted that the data in Fig. 4 represent the signal obtained from approximately 40 beads. The variation of the output signal has also been studied with respect to variation in the number of magnetic beads (ranging from 60 to 900) at the sensor area (data not shown). A lower output signal was observed with fewer beads or with beads placed further away from the coplanar waveguide (the pickup waveguide). Further, at the lowest number of beads measured (approximately 60 beads), the observed signal to noise ratio was approximately ten. These measurements characterize the sensitivity of FMR detection and will enable optimization

of the microwave waveguide design for single bead detection in subsequent studies. As reported in [14], single bead sensitivity may be achieved with optimization of the junction geometry, in particular, the spacing between the slotline (source) and CPW (pickup).

4.3. *Sensor area functionalization and characterization*

[Insert figure 5 here]

The method of photo-activated patterning was chosen to biotinylate the sensor area as it enabled better spatial control of the area to be functionalized compared to other methods such as microcontact printing. As discussed in Section 3.5.1, the silica-coated sensor area was functionalized with EZ-Link TFPA-PEG₃-Biotin, a photoactivable biotin containing the tetrafluorophenyl azide group. Upon UV irradiation, TFPA forms a highly reactive perfluoroaryl nitrene group that covalently bonds with the primary amino groups on the silica surface, previously introduced by treatment with 2% (v/v) APTS solution. The region where biotin is conjugated to the amine groups defines the active sensor area.

The photo-patterning protocol was verified by then treating the biotinylated area with fluorescein-neutravidin and imaging under a fluorescence microscope. As can be seen from Fig.

5b, the technique produces homogeneous biotin coverage at the sensor area.

4.4. *Demonstration of detection technique*

[Insert figure 6 here]

Streptavidin-coated beads were flowed in the microchannel over the biotinylated junction. Simultaneously, a *dc* current was applied to the CPW. The resulting magnetic field gradient attracted the beads to the tips around the CPW – coincidentally, also the region of highest sensitivity. The efficiency of biomolecular reactions in immunosensing techniques is typically limited by the diffusion rate of the molecular species. Applying a magnetic field gradient draws the beads from solution and enhances the likelihood of streptavidin-biotin interactions and thereby improves the binding efficiency. Fig. 6a shows the immobilized beads remaining on the junction after thorough rinsing with PBS. For comparison, the control device without biotin is also shown (Fig. 6b). As expected, no beads were retained after rinsing. The signal measured from the immobilized beads was plotted in Fig. 6c and is easily discerned from the control. These data demonstrate the proof-of-concept of inductive detection of magnetic labels using a sensor implemented with standard semiconductor processing techniques. Further experiments are underway to adapt the sensor for detection using antibodies and aptamers and to fully establish the analytical figures of merit for this sensor.

5. Conclusion

To summarize, a new microfluidic immunosensing technology using FMR-enhanced, inductive detection of magnetic bead labels has been demonstrated. The key advantage of this approach lies in the use of materials and processes compatible with and standard in integrated circuit manufacture. The sensor can thus be readily integrated with electronic circuitry for a low-cost, immunosensing platform amenable to a wide range of biosensing applications.

The variation of the output signal as a function of the location of magnetic beads on the sensor area was studied to determine regions of highest detection sensitivity. Proof-of-concept experiments were conducted using biotin-avidin binding. An integrated PDMS microfluidic channel was used to introduce streptavidin-functionalized magnetic beads. The resulting binding of the beads produced a distinct sensor output compared to a control. The use of magnetic fields to enhance the interaction of the beads with the biochemically functionalized surface was also demonstrated. The beads were attracted to the most sensitive regions of the sensor. This enhancement of the interaction rate between the analyte and sensor will be essential for the detection of very low concentrations of analyte. Experiments are currently under way to determine the ultimate chemical sensitivity of this approach.

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