A MAGNETIC FLOW CYTOMETER WITH INTEGRATED MICROFLUIDICS

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ABSTRACT

A chip scale flow cytometer realized in a standard CMOS process uses magnetic beads as labels. The material dependent complex susceptibility is used to differentiate between label classes for multi-target flow cytometry. The chip is embedded in a microfluidic cartridge and operation verified with an embryonic fibroblast assay.

INTRODUCTION

Flow cytometers are used to quantify cells or other particles in a biological sample such as blood and are indispensable tools in hematology, medical diagnostics, and food safety. Current optical flow cytometers use wavelength specific fluorescent molecules as tags. Although this approach achieves high throughput, the equipment is quite costly, too large for fieldwork, and suffers from background noise that requires calibration and extensive sample preparation. Replacing the fluorescent labels with superparamagnetic nanoparticles alleviates these problems, thereby enabling point of care flow cytometry [1].

In this paper, we present a flow cytometer that uses magnetic labels and a custom CMOS integrated circuit for self-contained detection. Details of the electronic circuit design are presented in [2]. This paper describes the theory of operation and microfluidic integration. The first section describes how susceptibility can be used to differentiate multiple magnetic label classes analogous to the wavelength division multiplexing commonly employed with fluorophores. This is followed by a description of the self-contained magnetic sensor amenable for integration in a standard IC technology, embedding in a microfluidic cartridge and experimental verification.

COMPLEX SUSCEPTIBILITY OF MAGNETIC NANOPARTICLES

Many applications of flow cytometry require establishing the relative counts of several distinct species such as CD3 and CD4 cells. Optical approaches meet this requirement with labels that fluoresce at different wavelengths. Magnetic labels instead rely on differences of the magnetic material properties.

The labels used in these experiments consist of superparamagnetic single-domain nanoparticles with 8nm-15nm diameter encapsulated in a polymer or silica matrix. The complex susceptibility [3]

$$\chi(\omega) = \chi'(\omega) - i\chi''(\omega)$$

of such an assembly of nanoparticles can be described in terms of its parallel and perpendicular components [4],

$$\chi(\omega) = -(\chi_{\parallel}(\omega) + \chi_{\perp}(\omega))$$

where, owing to their characteristics, $\chi_{\parallel}(\omega)$ and $\chi_{\perp}(\omega)$ are also referred to as the relaxation and resonant components of susceptibility.

Relaxation is an exponential function of nano-particle volume V , while resonance is primarily affected by the effective magnetic anisotropy constant K of the nanoparticle, a material property [4].

Either effect can, in principle, be used to distinguish between different labels, fabricated in the first case from nanoparticles of different size and different materials in the second case.

Figure 1 shows the simulated phase angle of the susceptibility as a function of frequency for 6nm and 8nm diameter nanoparticles, respectively made of the same material (magnetite, $\bar{K}=12 \times 10^3 \text{ J/m}^3$). The dotted lines apply to mono-disperse nanoparticles and demonstrate a phase difference of up to 28 degrees at 475 kHz that can be easily detected.

In practice, it is difficult to manufacture mono-disperse nanoparticles. The solid line represents the frequency response of an assembly of particles with a more realistic log-normal volume distribution with standard deviation $\sigma_V=0.3$ and log-normal K distribution with standard deviation $\sigma_K=0.58$. In this case the maximum phase difference between labels shrinks to 8.3 degrees, a value that is easily exceeded by particle-to-particle variations and is thus not suitable for differentiation.

Figure 1. Phase vs. frequency of relaxation component of complex susceptibility for mono-disperse (dotted) and particles with log-normal size and K distribution.

Unlike relaxation, magnetic resonance is only weakly sensitive to nanoparticle volume and is instead dominated by material properties. Figure 2 shows the frequency response of magnetic labels consisting of two different materials with magnetic anisotropy constant $\bar{K}=12 \times 10^3 \text{ J/m}^3$ (magnetite) with log-normal distribution ($\sigma_K=0.58$) and $\bar{K}=70 \times 10^3 \text{ J/m}^3$ (cobalt) with log-normal distribution ($\sigma_K=0.1$) respectively for identical particle volume distributions as before. At 1.2GHz, the phase difference is

greater than 70° thus enabling reliable classification.

Figure 2: Phase vs. frequency of resonant component of complex susceptibility for poly-disperse particles of mean diameter 6nm and 8nm with log-normal distribution for two different materials.

SENSOR DESIGN

Identification of different label classes based on magnetic resonance requires a sensor capable of magnetizing at frequencies in excess of 1GHz to measure not only the magnitude, but also the phase of the response. On-chip coils fabricated in standard CMOS processes are ideally suited for this task. The sensor consists of a polarization coil driven by an AC waveform at the detection frequency to generate a field that is uniform in the detection area.

A pair of secondary coils is used to detect the presence and phase response of a magnetic particle as shown in Figure 3. Without a particle present, magnetic field in both coils is identical and produces equal responses. Series connection with opposite winding directions results in a net zero response.

The presence of a bead modifies the coupling of the polarization field. By choosing the size of the pick-up coils larger than the particles (approximately $10\mu\text{m}$ for typical cells), the change is localized to one of the two coils and the resulting imbalance produces a voltage at the sensor output. In the actual design slightly larger coils with dimension $30\mu\text{m}$ by $30\mu\text{m}$ are chosen as a compromise between detection area and sensitivity.

A cell decorated with magnetic particles and passing over the two detection coils results in a bipolar response. For clarity, the GHz modulation is omitted from the graph. Demodulation occurs on-chip with a synchronous quadrature receiver [2].

INTEGRATION OF CMOS CHIP WITH MICROFLUIDICS

Figure 5 shows the microfluidic setup. The surface of the chip is exposed to a $50\mu\text{m}$ tall and $200\mu\text{m}$ wide channel that guides labeled cells over the detection area. Although the magnetic labels do not require optical access, a transparent setup has been chosen to aid debugging. The setup can be easily disassembled for cleaning. In the actual application, a disposable cartridge is

preferred.

Figure 3: Schematic showing the secondary pick-up coil for detecting the change in magnetic flux due to the presence of a cell decorated with magnetic labels.

Figure 4 shows the fabrication process starting with a standard printed circuit board (PCB) used for mechanical support and electrical interconnects. An adhesive tape temporarily holds the $2.5 \times 2.5\text{mm}^2$ CMOS chip in place inside a cavity in the PCB and avoids steps in the final microfluidic channel that would result in unwanted turbulence. In the next step, the cavity is filled with epoxy and allowed to cure for 1 hour. The tape is then removed from the PCB surface. The chip is then wire-bonded to the PCB, and the wirebonds are protected with Crystalbond (Structure Probe, Inc.).

The PDMS micro-channel is fabricated with an SU8 mold (Stanford Microfluidics Foundry). PDMS (Sylgard 184) was mixed with the polymerizing agent in the ratio 10:1, degassed for half an hour and poured on the mold and allowed to cure at 60°C for 3 hours. The PDMS is cut to appropriate dimensions.

The PDMS micro-channel is placed on the chip and aligned visually to the detection coil on the chip using a microscope. A cavity is made in the PDMS micro-channel to accommodate the wirebonds. A glass slide applies uniform pressure and compression seals the channel against the PCB using acrylic supports. Figure 5 schematically shows the device after complete integration.

Figure 4: Steps to integrate the CMOS chip with PDMS micro-channel.

Figure 5: Schematic showing the cross-section of the integrated flow cytometer.

MAGNETIC LABELING OF CELLS

Device operation is demonstrated with mouse embryonic fibroblasts (mEFs) conjugated to magnetic labels as shown in Figure 6. The mEFs are obtained from the Tissue Culture Facility at UC Berkeley. The cells are grown in standard DMEM (Dulbecco's Modified Eagle Medium) with added penicillin-streptomycin and 10% FBS (fetal bovine serum). Cells are maintained at 37°C and 5% CO₂ in air. An antibody buffer consisting of 1X PBS, 0.5 % BSA, and 2 mM EDTA was prepared fresh before the experiment.

Once cells achieve 75-80 % confluence, the cells are labeled with biotinylated mouse CD-29 primary antibody [Biolegend] at a concentration of 0.25μg (from a stock solution of 0.5 mg/ml) per 10⁶ cells. Following a 15 minute incubation at 37°C, the cells are washed twice with buffer. Then, 2μl of streptavidin coated

Dynabeads [5] (from a stock solution of 4x10⁸ beads/ml) per 10⁶ cells is diluted in buffer, added to the cells as a secondary label for the CD-29, and incubated at 37°C for another 15 minutes. Following incubation, the excess beads are washed off with buffer three times with rigorous mixing. It is imperative that cells be visualized under a microscope to ensure that excess beads are removed as they may overwhelm the cell detection by the chip. The cells are then treated with 0.25% trypsin for 5 minutes at 37°C. The trypsin was neutralized with calcium-free PBS; necessary because calcium would allow cells to aggregate. These cells are harvested and run through the cytometer within 2 hours of harvesting.

Figure 6: Magnetic labeling of mouse stem cells (mEFs).

EXPERIMENTAL SETUP

The setup for carrying out the experiment is shown in Figure 7. The micro-channel is initially flushed with DI water to remove any contaminants and air bubbles. The sample containing the labeled cells is connected to the inlet port of the micro-channel. A syringe pump controlled by custom software is connected at the output port of the micro-channel and applies suction to carry the cells through the micro-channel. The flow rate for measurements is about 1mm/s. In a final design this function can be integrated (e.g. using a micro-pump or degas-driven flow [6]).

The circuits on PCB provide the supply and biasing required for the CMOS chip. The output of the chip is buffered and amplified and then digitized with an NI PXIe-5122 data acquisition system. Custom software implemented in NI LabView digitally processes the signals. A microscope is used to help visualize the flow of labeled cells over the sensor coil.

Figure 7: Experimental setup for flow measurements.

MEASUREMENT RESULTS

Figure 8 shows the completed flow cytometer. The inset shows the microfluidic channel on the surface of the CMOS chip with a

magnetically labeled cell flowing over the sensor. The measured bipolar response of the chip to Dynabeads is shown in Figure 9. The measured response to labeled cells is presented in [2]. The throughput of the system is 50 beads/s or 20 labeled cells/s, which is comparable to the point-of-care flow cytometer described in [1]. The labeled cells are detected with an SNR of 16dB. Figure 10 shows the results of a phase measurement of iron oxide and cobalt beads (Ocean Nanotech). The measured phase angle is $\langle \phi_{\text{Dynabead}} \rangle = 91^\circ$ ($\sigma = 9^\circ$) and $\langle \phi_{\text{Cobalt}} \rangle = 56^\circ$ ($\sigma = 6^\circ$) and is independent of magnitude. This is expected since Dynabeads made of iron oxide has smaller $\bar{K}$ compared to cobalt.

Figure 8: Fabricated flow cytometer cartridge (top) and sensor chip (bottom).

Figure 9. Measured time domain response of the flow cytometer for Dynabeads.

Figure 10: Phase measurement of iron oxide and cobalt beads. A clear differentiation of the two label classes can be seen.

CONCLUSIONS

Magnetic labels allow the entire detection process of a flow cytometer to be realized on a millimeter-sized electronic circuit fabricated in a CMOS process, allowing for a compact and economical implementation that is particularly attractive for point-of-care applications. By measuring not only the magnitude, but also the phase of the induced magnetic field, the chip is capable of differentiating between magnetic labels fabricated from different materials.

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