

A GMR-based Magnetic Flow Cytometer Using Matched Filtering

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Abstract—In this work, signal processing techniques such as matched filtering and cross-correlation were applied to improve the detection efficiency in magnetic flow cytometry measurements with a SNR as low as 4.5 dB, a 3-fold improvement over thresholding alone. A multi-stripe GMR sensor was fabricated to improve the discrimination between magnetic nanoparticles (MNPs) and interference/noise such as motion artifacts. Time-of-flight between successive sensors in the array enables multi-parametric and hydrodynamic analysis of cells. Detection of 4.5 μm MNPs and polymer microbeads decorated with MNPs, serving as a biomimetic model of cells, shows proof-of-principle. This setup was then optimized for future cell detection experiments.

Keywords—GMR; Flow Cytometer (FCM); Matched Filter

I. INTRODUCTION

A flow cytometer (FCM) is an essential tool in hematology for quantitative analysis of cells with applications including identifying prognostic indicators for cancer, HIV, and other time-dependent biomarkers of disease activity [1]–[3]. Optical measurement techniques, such as forward scatter (FSC), side scatter (SSC), and fluorescence are currently considered the gold standard for multi-parametric analysis of heterogeneous cell populations [4]. However, such instrumentation requires complex optics, lasers, and photodetectors making it hard to translate to the point-of-care (POC). Furthermore, optical FCMs require extensive sample preparation which involves cell lysis or matrix purification to properly detect cells/cell surface receptors (e.g., CD4) from crude samples with a substantial optical background. As such, optical FCMs remain grounded in centralized laboratories with well-trained personnel resulting in long turnaround times and out of reach for routine monitoring by individuals or in office check-ups.

An alternative approach involves substituting the optical label with a magnetic tag and using a magnetic sensor instead of an optical detector [5]. This configuration has many benefits: 1) biological samples have no magnetic background eliminating problems such as photobleaching and auto-fluorescence commonly encountered in optical measurements, 2) the sample preparation can largely be eliminated, and 3) magnetic biosensors can be miniaturized, which also improves their sensitivity [6]. As such, magnetic FCMs have been of keen interest to the community for POC measurements [7]–[9].

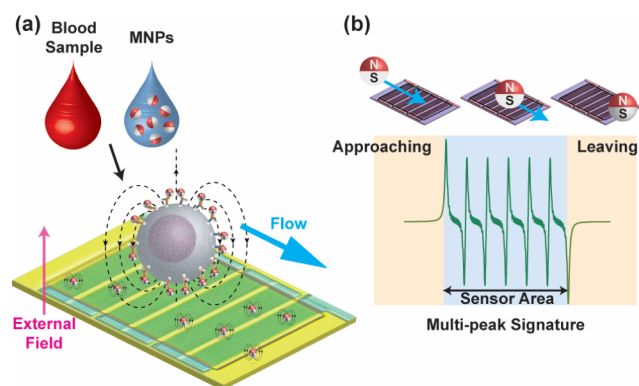


Fig. 1 Illustration of a GMR-based FCM: (a) principle of operation (not to scale) where MNPs bind to cells and flow over magnetic sensors and (b) corresponding magnetic signature generated by a multi-stripe sensor.

In this work, we developed a GMR-based FCM using matched filtering to improve the SNR and allow detection of different types of magnetic labels (Fig. 1a). As the magnetic label flows over the sensor, a change in resistance is induced. The carefully designed sensor layout creates a characteristic signature from the MNPs, as shown Fig. 1(b), thus enabling multi-parametric labeling like optical FCMs. Furthermore, the array of sensors can extract the time-of-flight (ToF), which can be used as a proxy for the size and hydrodynamic volume of the cell. In this proof of concept work, 10 μm polymer beads were used as a biomimetic model. Complexes decorated with MNPs were flown over the GMR sensor array using microfluidic channels and detected with a SNR as low as 4.5 dB due to the processing gain afforded by the matched filtering.

II. GMR-BASED MAGNETIC PLATFORM

A. GMR SV Sensors and Microfluidics

Each giant magnetoresistive spin-valve (GMR SV) sensor chip (MagArray, Inc.) has 80 individually addressable sensors arranged in an 8×10 matrix with a nominal resistance, R_0 , of 1.7 k Ω and a magnetoresistance ratio (MR) of 11.5%. Each sensor is $120 \times 120 \mu\text{m}^2$ and the multi-stripe geometry (Fig. 1) was designed to create a distinct magnetic signature used for matched filtering (described later). Two techniques were used to realize the microfluidics. For the non-photolithographic process, a poly(methyl methacrylate) (PMMA) sheet was laser cut and integrated with double-sided adhesive (3M™ OCA 8211) to

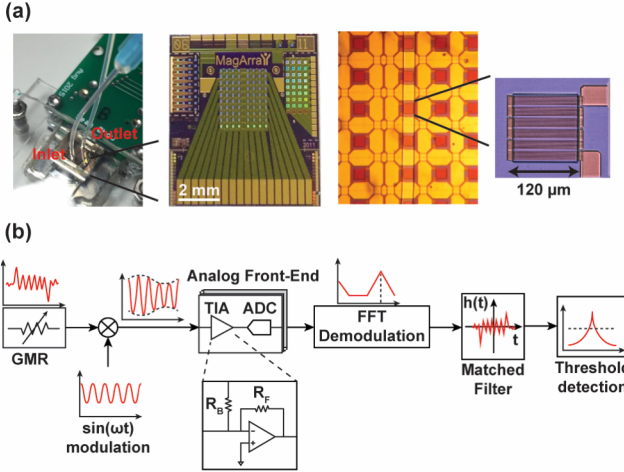


Fig. 2 (a) Photograph of the system and zoomed-in view (from left to right) of the sensor chip, microfluidic channel, and an individual sensor. (b) Block diagram of the detection scheme.

form 250 μm wide, 25 μm high microchannels over the sensors. For the photolithographic process, standard PDMS fabrication techniques were used to form 59 μm high channels with 120 μm width (Fig. 2a). GMR chips were placed in UV-ozone (HELIOS-500, UVOTECH) for 30 minutes prior to bonding with the PDMS microfluidic channel to improve sealing. Lastly, PDMS channels were aligned and cured for 1 hour at 75 °C. Both setups have 8 sensors per channel. The fluidic biochip plugged into a connector on the PCB with a 19×19×1.6 mm NdFeB permanent magnet (BCC2, K&J Magnetics, Inc.) mounted below the sensor array (Fig. 2a). The magnet generated a 0.13 T out-of-plane field that magnetized the superparamagnetic nanoparticles. The inlet and outlet of the PDMS channel were mechanically milled and connected to a reservoir and syringe pump (NE-300, New Era Pump Systems), respectively.

B. Magnetic Nanoparticles and Polymer Microspheres

Superparamagnetic MNPs, Dynabeads (M-450) and Bio-Ademeads, were used in all experiments with diameters of 4.5 μm and 200 nm, respectively. 10 μm ProActive biotin coated polymer microspheres (CP10N, Bangs Laboratories Inc.) were used for proof of principle experiments as a mimic of cells. Complexes were constructed by incubating 0.05× ProActive microspheres and 10× Bio-Ademeads for 1 hour at 4 °C.

C. Interface

The GMR sensors were excited by a 1 V_{pp} sinusoidal source at 7 kHz generated by a data acquisition card (NI PCIe-6361) as shown in Fig. 2(b). The resulting current was amplified by a transimpedance amplifier (TIA) implemented using an OpAmp (AD 8655) with resistive feedback ($R_F = 42.2$ kΩ). A bleed resistor ($R_B = 1.7$ kΩ) was used to discharge the non-magnetoresistive portion of the current and avoid saturating the TIA, thus improving the gain by 28 dB. The TIA output was sampled at 125 kSps and processed in LabVIEW using a Fast Fourier Transform (FFT) to demodulate the signal ($BW = 1$ kHz). The demodulated signal was passed through a matched filter where the signal was convolved with the time-reversed impulse response of the expected signature (derived from micromagnetic simulations). A detection event was declared

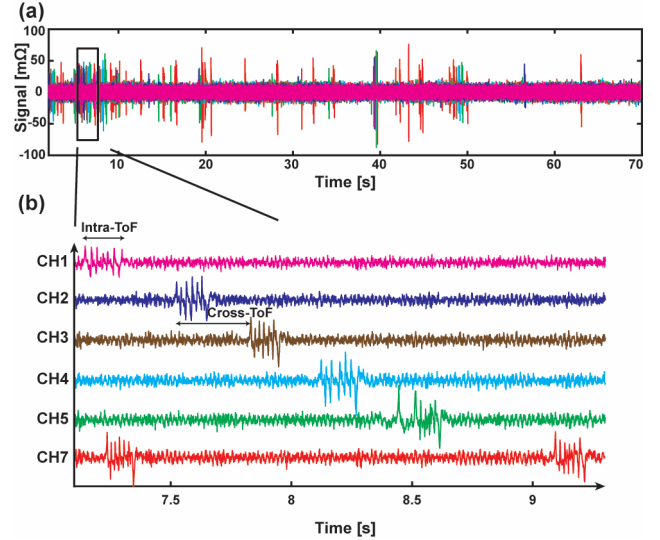


Fig. 3 FCM measurements showing (a) detection of 4.5 μm MNPs with (b) ToF sequencing through the sensor array.

when the matched filter output exceeded $5\times$ the noise level. The input-referred noise of the system was measured to be 3.5 mΩ_{RMS} and spectrally white around the excitation frequency.

III. RESULTS AND DISCUSSION

A. ToF Measurements

Fig. 3(a) shows raw measurement data (no matched filtering) collected while flowing 4.5 μm MNPs over the sensors at 5 μL/min. Visually, several events can be seen from the unprocessed data, but zooming in an event that occurred at 7.5 seconds most clearly illustrates the operation of the system. The distinct signature from a MNP is first observed on CH1 and then successively on each of the subsequent channels (CH2-7) as the MNP flows through the microfluidic channel (Fig. 3b). From this data, both the intra-sensor ToF (time between peaks within a signature) and the cross-sensor ToF (time between signatures of adjacent channels) can be calculated. The intra-sensor ToF was 109 ms and the cross-sensor ToF was 290 ms corresponding to a velocity of 1.10 mm/s. The amplitude of the signature can be used to calculate the vertical position of the MNP and the number of MNPs in the case of an aggregate structure. It should be noted that this requires micromagnetic simulations due to the non-linear interaction of multiple MNPs. With the current sampling rate, this system can handle velocities up to 5 mm/s before the shape of the signatures is distorted.

B. Matched Filtering

While some of the events are clearly visible to the naked eye, the signal further decreases when smaller MNPs are used or as the vertical distance to the sensor (flow height) is increased. Both matched filtering and cross-correlation analysis were used to improve the SNR and enhance the detection efficiency. Whereas many previous designs used only a single stripe sensor resulting in a simple bipolar signature [5], the complicated multi-peak signature here (Fig. 3b) provides a more reliable and robust matching sequence reducing the minimum SNR from 14 dB to 4.5 dB.

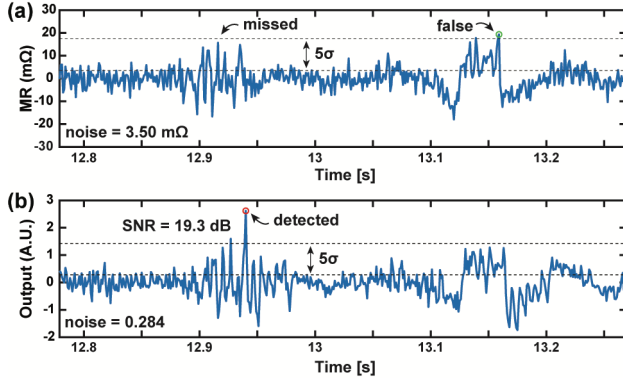


Fig. 4 Measured data showing (a) missed detection using only thresholding and (b) correct detection using matched filtering.

After finding the signature sequence on any of the channels, the detected signature (not the expected one) is used as a template to compare against all other channels for correlation within a window based on the pump rate. In a relatively small time window, the flow rate, MNP distance to the sensor, and other slowly-changing environmental parameters can be regarded as constants. Therefore, each of the channels produces the same signature, but delayed in time based on the velocity. A majority voting algorithm with the eight sensors is used to reduce uncorrelated noise and declare a detection event.

To illustrate the signal processing, Fig. 4(a) shows a snippet of measured data. Simply thresholding at 5σ ($\text{SNR} = 14 \text{ dB}$) results in missing the signature; however, when applying the matched filter (Fig. 4b), the event is clearly visible at 12.95 s. It should be noted that though the noise floor increases after matched filtering, the signal increases even more. For example, a motion artifact caused a large impulse response at 13.15 s which could be easily be counted as a false detection with thresholding. However, since this impulse does not possess a signal-like signature, the matched filtered output is kept within the 5σ threshold and it is correctly rejected.

C. Complex Detection

Fig. 5 shows measurement results from various samples. There is no appreciable signal when flowing PBS or unbound Bio-Adembeads through the channel, although the noise floor of Bio-Adembeads is visually higher than that of PBS. When complexes of $10 \mu\text{m}$ polystyrene beads conjugated to Bio-Adembead MNPs were injected into the channel, many detection events were detected (only the first 10 are marked for clarity). Only the complexes with more than one MNP are detected as the 200 nm MNPs alone result in a signal that is below the noise floor (unlike the larger M-450 MNPs). These particles have also shown to not form aggregates in isolation and thus do not generate a detectable signal when they are not conjugated in aggregate to cells meaning that the unbound MNPs do not need to be removed prior to measurement. Since the pumping rate is very fast ($50 \mu\text{L}/\text{min}$), the hydrodynamic drag force on the MNPs dominates other forces including magnetic force allowing the complex to flow through the middle of the channel.

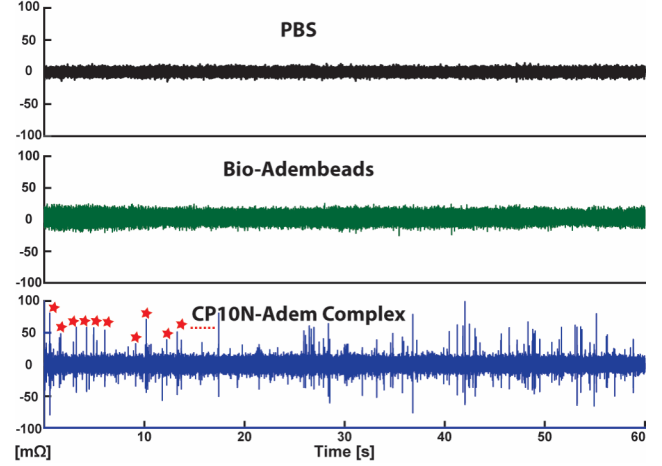


Fig. 5 FCM measurements of PBS, unconjugated MNPs, and complexes.

IV. CONCLUSION

A GMR-based FCM is proposed to integrate with a multi-stripe sensor layout and signal processing to improve the detection efficiency. The multi-stripe layout generates a characteristic signature that is detected using a matched filter allowing discrimination between different types of magnetic labels. The sensor array allows ToF measurement for multi-parametric analysis. Biomimetic experiments were validated with MNP decorated polymer microbeads and optimized for future experiments with real cells. This GMR-based FCM offers an alternative to optical FCM with increased portability and rapid “sample-to-answer” measurement capability.

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