Toward a magnetoresistive chip cytometer: Integrated detection of magnetic beads flowing at cm/s velocities in microfluidic channels

J. Loureiro, R. Ferreira, S. Cardoso, P. P. Freitas, J. Germano, C. Fermon, G. Arrías, M. Pannetier-Lecomte, F. Rivadulla, and J. Rivas

1INESC-MN/Institute for Nanosciences and Nanotechnologies, R. Alves Redol 9, 1000-029 Lisbon, Portugal and Instituto Superior Técnico, Universidade Técnica de Lisboa, 1049-001 Lisbon, Portugal
2INESC-ID, R. Alves Redol 9, 1000-029 Lisbon, Portugal and Instituto Superior Técnico, Universidade Técnica de Lisboa, 1049-001 Lisbon, Portugal
3DSM/IRAMIS/SPEC, CEA Saclay, 91191 Gif-sur-Yvette Cedex, France
4Instituto de Química, Universidade Federal Fluminense, Rio de Janeiro, Brazil

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This work describes an integrated device comprising microfluidic channels and incorporated spin-valve sensors sensitive enough to count, determine the magnetic orientation, flowing height, and speed of single micron-sized magnetic beads moving with velocities of 8–35 mm/s. Sensor signals of $3–100 \, \mu V_{p-p}$ correspond to bead moments at different directions indicating a physical rotation of the beads and a slow response (seconds) of the bead moment to magnetizing field changes. © 2009 American Institute of Physics [DOI: 10.1063/1.3182791]

Over the past decade, the drawbacks of conventional flow cytometers have encouraged efforts to take advantage of microfabrication technologies and advanced microfluidics to achieve smaller, simpler, more innovative, and less expensive instrumentation. Magnetoresistive sensors can be used to replace the external solid-state devices (diode laser, PIN photodiode) that make these systems complex and be easily integrated within microfluidic channels to detect/count magnetically labeled cells. These sensors have been integrated in biochip platforms used for biomolecular recognition detection, measuring the fringe field created by magnetic particles (MP).

First attempts at MP dynamic detection were made either with ferrofluidic droplets (tens of microns long) moving inside microfluidic channels with cm/s velocities, or with single MP manipulated over the sensor using current lines actuation involving slowly moving particles ($\mu$m/s). This paper describes an integrated cytometer that can monitor in real time the full distribution of velocities, height, and magnetization orientation, for ensembles of MPs moving with velocities up to cm/s within microfluidic channels 14 $\mu$m high.

The microfabricated device described in this paper has been designed to incorporate $2.5 \times 40 \, \mu$m$^2$ spin-valve (SV) sensors (width $\times$ distance between electrical leads) integrated with 150 $\mu$m wide and 14 $\mu$m thick microfluidic channels. The channels are made of polydimethylsiloxane (PDMS) using a standard micromolding technique.

The sensing element of the device is a SV, microfabricated with a yoke-shape geometry over Si passivated with 500 $\AA$ of Al$_2$O$_3$ deposited by rf magnetron sputtering. Top pinned SV sensors were fabricated by ion beam deposition (Nordiko3000 system) with the following structure (thickness in angstroms): substrate/Ta 20/Ni$_{50}$Fe$_{20}$ 25/Co$_{20}$Fe$_{20}$ 25/Cu 20/Co$_{10}$Fe$_{20}$ 25/Mn$_{76}$Ir$_{24}$ 60/Ta 20/TiW(N$_2$) 150 (compositions in at. %). The 150 $\AA$ thick Ti$_{10}$W$_{90}$ passivation layer was deposited by magnetron sputtering. A 3 mT magnetic field was applied during deposition to induce the free layer and pinned layer easy axis with parallel anisotropies. The transfer curve of the sensors is shown in Fig. 1(a) (1 mA bias current).

The sensors were passivated with sputtered Al$_2$O$_3$ (1000 $\AA$) and SiO$_2$ (7000 $\AA$) to protect against corrosion caused by the fluids and to enable the chemical bonding with the PDMS. The microfabricated wafer was diced into individual dies and wire bonded to a printed circuit board. To obtain an irreversible bonding between the PDMS and the SiO$_2$ surface, the chip was cleaned for 30 min with 28 mW/cm$^2$ ozone plasma, with 5 mm separation from the UV light (UVO Cleaner, Jelight, USA) prior to the activation step.

FIG. 1. (Color online) (a) Transfer curve of a SV sensor with an average MR of 7% and a sensitivity of 5 V/T. The inset shows the simulated sensor output according to the position $(x,0,z)$ of a flowing MP vertically magnetized by a 90 mT field. Maximum signals of 6 $\mu V_{p-p}$ are expected for MPs at $z=10 \, \mu$m and 40 $\mu V_{p-p}$ at $z=3 \, \mu$m. (b) Picture of the microchip bonded and aligned with the microfluidic channel and with microcapillaries mounted.

$^a$Electronic mail: jloureiro@inesc-mn.pt.
Both surfaces (chip SiO₂, passivation layer and PDMS channels) were then oxygen-plasma activated (68 mW/cm², 120 mTorr, 10 in., 20 (SCCM) (SCCM denotes standard cubic centimeters per minute at STP) O₂, with a LAM Rainbow oxide etcher) and the PDMS channels were aligned to the sensors before the irreversible bonding is achieved [Fig. 1(b)].

In order to measure the small signals arising from the flowing MPs, a two stage signal amplifying system was used. A home made very low noise preamplifier [1.2 nV/sqrt(Hz)] incorporating a high pass filter at 3 Hz, a low pass filter at 6 kHz, and a gain of 56 dB followed by a commercial Stanford amplifier (SR560) (set with a high pass filter at 100 Hz, a low pass filter at 3 kHz, and a gain of 14 dB) have been connected to the SV sensor, leading to a 70 dB amplification of the MP signals. The output is then acquired on a computer through a commercial digital to analog conversion board (Data Translation, 16 bit DAC) using an acquisition program that allows continuous acquisition up to a sample rate of 20 kHz. With this setup, the total rms noise of the measurement is 1 μV for a bandwidth of 3 kHz and a sensor biasing current of 1 mA. This corresponds to field equivalent rms noise of 215 nT. The sampling frequency of the acquisition has been chosen to be 3 kHz in order to provide a good temporal resolution of the signal.

The device was tested injecting 2 μm (diameter) MPs (Micromer—M, Micromod, Germany) diluted in a phosphate buffer solution (100 mM, pH 7.4). Since these micron-sized MPs are formed of polystyrene-co-maleic acid with microcones on the surface filled with superparamagnetic FeOx nanoparticles (NPs), a 90 mT vertical external field (permanent magnet under the chip) is applied to induce a magnetic moment. The inset of Fig. 1(a) shows the simulated sensor output, while a vertically saturated MP moves at a height z (3, 7, and 10 μm) over the sensor (which is biased with 1 mA).

Figure 2(a) shows the raw signal from a burst of MPs injected into the microchannel while Fig. 2(b) shows the same data after digital filtering it with a high pass filter with a cutoff frequency of 150 Hz (Equiripple FIR algorithm in MATLAB). The filtering procedure reduced the rms noise from 1 to 0.3 μV. A home made software was used to perform a statistical analysis of the MPs physical characteristics when flowing inside the microchannels (velocity of the particle (v), height of the particle in the microchannel (z), and magnetization angle with respect to the vertical direction (θ)).

A least square regression method has been used to assign each experimentally measured pulse to a point in the phase space defined by the three unknown variables (v, z, and θ). This method, implemented using MATLAB language, starts by calculating the exact pulse shape for each point of the phase space and then comparing it to the experimental one. The velocity parameter was allowed to vary between 5 and 35 mm/s (with 1 mm/s step), the height between 1 and 13 μm (with 1 μm step), and θ between 0° and 360° (with 5° step).

Figures 3(a) and 3(b) shows the distribution of measured signal pulses (represented with solid circles) corresponding to two different orientations of the bead moment and different velocities, when passing over the sensor. The solid line corresponds to the pulse reconstruction using the best fitting parameters (shown in the inset table). The bipolar pulse in Fig. 3(a) results from a MP with near vertical magnetization direction, while the unipolar pulse [Fig. 3(b)] corresponds to a MP with almost horizontal magnetization.

Figure 4(a) shows the distribution of the beads’ magnetization angle, with a mean clockwise angle of 17° with respect to the vertically applied magnetizing field and a standard deviation of 27°. Figure 4(b) shows the distribution of particle’s heights. Most of the MPs (82%) are in the lower half of the channel, meaning they will be imparted a clockwise rotation from the velocity profile of the fluid in z direction. Figure 4(c) shows the velocity distribution with an average value of around 22 mm/s.

FIG. 2. (Color online) (a) Raw data acquired at 3 kHz, corresponding to a burst of particles flowing on top of the sensor. (b) Same data as shown in (a) after digital filtering it. The exploded view shows an example of two peaks corresponding to two flowing particles, the first one having 10 points available to the reconstruction analysis and the second one 8 points.

FIG. 3. (Color online) (a) Bipolar pulse observed from the passage of a bead with a quasi-vertical (15°) moment, a velocity of 23 mm/s and a height of 6 μm; (b) unipolar pulse caused by the passage of a bead with close to horizontal (−110°) moment, with a velocity of 17 mm/s and a height of 6 μm.
To understand why the magnetization of the MPs is not vertical (but instead 17°) and has a wide standard deviation, a detailed characterization of the MPs was performed showing that they are composed of strongly interactive NPs. Therefore two different magnetic relaxation regimes are observed: a fast one (Néel type) usually associated to a local relaxation of the magnetic moment toward the easy axis of the NPs, and a slow one which is characteristic of the MP relaxation in the fluid or characteristic of strongly interacting NPs with blocking temperatures near room temperature (spin glass behavior).

ac susceptibility measurements in dried and liquid samples were performed with a Physical Property Measurement System (Quantum Design) with 1 mT ac field. Figure 5 shows the imaginary component of the complex susceptibility for beads within a liquid carrier (circles). Two maxima are visible, one broad peak at high frequencies (responsible for a fast magnetic relaxation mechanism: \( \sim 15 \mu s \)) and another one at low frequencies, already beyond the limit of the measurement. For dried particles (squares), again two maxima appear for the same frequencies, although with smaller intensity.

The observed MPs angle distribution can now be explained considering the slow relaxation time mechanism and a random distribution of the blocked NPs magnetization. The SVs are measuring the remanent magnetic moment of the micron-sized MPs which did not have enough time to relax due to the strong interactions of some NPs. However the average angle is around the equilibrium position (vertical direction) since most of the magnetic NPs relaxed through the fast relaxation mechanism. The average distribution around 17° may be explained due to the clockwise rotation of the MPs due to the velocity profile of the liquid inside the channel.

In conclusion, an integrated microfluidic system incorporating SV sensors has been fabricated allowing single particle detection and counting for particle velocities up to 35 mm/s. Furthermore, the speed, the flowing height as well as the magnetic orientation of each particle has been determined.

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