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Hybrid integration of scalable mechanical and magnetophoretic focusing for magnetic flow cytometry

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ABSTRACT

Time-of-flight (TOF) magnetic sensing of rolling immunomagnetically-labeled cells offers great potential for single cell function analysis at the bedside in even optically opaque media, such as whole blood. However, due to the spatial resolution of the sensor and the low flow rate regime required to observe the behavior of rolling cells, the concentration range of such a workflow is limited. Potential clinical applications, such as testing of leukocyte function, require a cytometer which can cover a cell concentration range of several orders of magnitude. This is a challenging task for an integrated dilution-free workflow, as for high cell concentrations coincidences need to be avoided, while for low cell concentrations sufficient statistics should be provided in a reasonable time-to-result. Here, we extend the spatial bandwidth of a magnetoresistive sensor with an adaptive and integratable workflow concept combining mechanical and magnetophoretic guiding of magnetically labeled targets for in-situ enrichment over a dynamic concentration range of 3 orders of magnitude. We achieve hybrid integration of the enrichment strategy in a cartridge mold and a giant-magnetoresistance (GMR) sensor in a functionalized Quad Flat No-Lead (QFN) package, which allows for miniaturization of the Si footprint for potential low-cost bedside testing. The enrichment results demonstrate that TOF magnetic flow cytometry with adaptive particle focusing can match the clinical requirements for a point-of-care (POC) cytometer and can potentially be of interest for other sheath-less methodologies requiring workflow integration.

1. Introduction

Functional cell diagnostics at the bedside requires an integrated and effortless workflow for results within a few minutes. However, the concentration of e.g. immune cells in a whole blood sample can vary over orders of magnitude, which is a significant challenge for point-of-care diagnostics (POC). With fluorescence flow cytometers, high-throughput is usually achieved with a sheath-flow based method and several preanalytical steps to minimize coincidences and background signal. The step towards POC, however, remains challenging, which is mainly due to the optical workflow, which requires the lysis of erythrocytes to bypass the optical opacity of whole blood and the removal of fluorescence excess markers by trained users (Shapiro, 2003; Greve et al., 2006; Einwallner et al., 2013; Robinson and Roederer, 2015).

To overcome the resulting complexity of an optical read-out, alternative magnetic methods with non-optical detection of biological targets based on magnetoresistive sensing mechanisms with either static (Osterfeld et al., 2008; Schotter et al., 2009; Gaster et al., 2011; Lin et al., 2014; Bechstein et al., 2015; Huang et al., 2017) or dynamic approaches (Loureiro et al., 2011; Issadore et al., 2012; Melzer et al., 2012; Helou et al., 2013; Fernandes et al., 2014; Kim et al., 2015; Reisbeck et al., 2016) have emerged (Lin et al., 2017). Herein, immunologically functionalized magnetic particles at the nanoscale replace fluorescent labels used in optical flow cytometry (Lee et al.,

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2015). Thus, magnetic flow cytometers for single cell detection overcome the restrictions of optical flow cytometers, such as absorption of the scattered light by the cellular background or the need for highly diluted samples, due to the inherently low magnetic moment of biological samples (Issadore et al., 2014).

Despite the great potential for miniaturization and integration of highly sensitive magnetoresistive elements, the step towards an integrated device for quantitative POC applications is challenged by two aspects. First, the integration of the fluidic transport and particle enrichment onto a cm^2 Si chip containing the sensing elements prevents low-cost, disposable bedside testing (Loureiro et al., 2011; Issadore et al., 2012; Melzer et al., 2012; Helou et al., 2013; Fernandes et al., 2014; Kim et al., 2015; Reisbeck et al., 2016). Second, similar to fluorescence flow cytometry (Keij et al., 1991; Wersto et al., 2001) and impedance sensing (Wynn and Hounslow, 1997; Kammel et al., 2012), quantitative magnetic information on a single particle level is masked by coincidences occurring at particle concentrations exceeding an instrument specific threshold. Thus, without prior knowledge of the particle concentration, an additional dilution step of the sample becomes necessary to avoid coincidences, while sufficient statistics should be provided for analysis of samples with low cell concentration.

In previous proof-of-concept studies the fluidic interconnection of mm^2-sized sensor chips to peripheral fluidic components has been presented using rather complex fabrication and alignment strategies of multiple Polydimethylsiloxane (PDMS) layers (Wu et al., 2010; Muluneh and Issadore, 2014). However, effortless hybrid integration of an all-magnetic workflow incorporating the singulation of magnetic targets in a clinically-relevant concentration range covering various log-scales into an injection-molded low-cost fluidic platform for quantitative POC testing has not been explored yet.

In our previous work we discussed a quantitative magnetic flow cytometry approach, which utilizes cell rolling for precise volumetric measurements and analysis of immunomagnetic binding capacity on a single particle level (Reisbeck et al., 2016). However, quantitative analysis of the magnetic fingerprint of single particles is limited due to the spatial resolution of the sensor and thus coincidences at high particle concentrations. At low particle concentrations, the time-to-result for sufficient statistics impedes bedside application. Here, we extend our previously reported magnetic flow cytometry workflow by an adaptive in-situ enrichment and present effortless hybrid integration of an all-magnetic workflow. We combine in-situ enrichment by means of mechanical chevrons and magnetophoretic forces to cover variable particle concentrations without the need for sample dilution steps and thus buffer reservoirs on a cartridge. We designed and integrated a functional package housing a magnetoresistive half-bridge sensor in a credit card-sized injection molded device, which comprises the mechanical focusing structures for adaptive in-situ enrichment. Furthermore, we show that with time-of-flight (TOF) probing of the magnetic fingerprint, accurate quantitative information on a single particle level can be obtained. Once mechanical and magnetophoretic focusing and TOF analysis is performed, magnetic particles are captured in an integrated cavity and can be extracted with diminished background for subsequent analysis.

2. Experimental

2.1. Detection mechanism and spatial resolution of the quantitative magnetic flow cytometer

The schematic in Fig. 1a illustrates the detection principle of our magnetic flow cytometry approach, allowing for quantitative non-optical probing of the magnet stray field of single magnetic particles in a highly reproducible manner. The magnetoresistive sensor is incorporated into a microfluidic channel and consists of two $2 \times 30\ \mu m^2$ giant magnetoresistance (GMR) sensors arranged transversely to the laminar flow direction of the magnetic particles in a Wheatstone half-bridge configuration. The sensor matrix is positioned precisely over the center of a permanent magnet, which pulls the magnetic particles towards the substrate surface and magnetizes the target particles orthogonal to the sensor plane (Hayden et al., 2016). For a single magnetic particle rolling over the sensor half-bridge at effectively zero distance a characteristic four-peak pattern is observed. By analyzing the normalized integral of the sensor signal, given by the sum of the absolute integral of each half-wave, we derive quantitative single particle information, such as volumetric information and immunomagnetic binding capacity. For TOF calculations, we analyze the characteristic sensor signal within its margins, defined as the starting and end positions where the signal increases above its baseline value by 25% and then drops to 25% of its maximum amplitude. Hence, the spatial resolution of the sensor is defined by the dimensions of the signal pattern arising from a single magnetic particle.

We evaluated this quantitative approach with respect to cross-

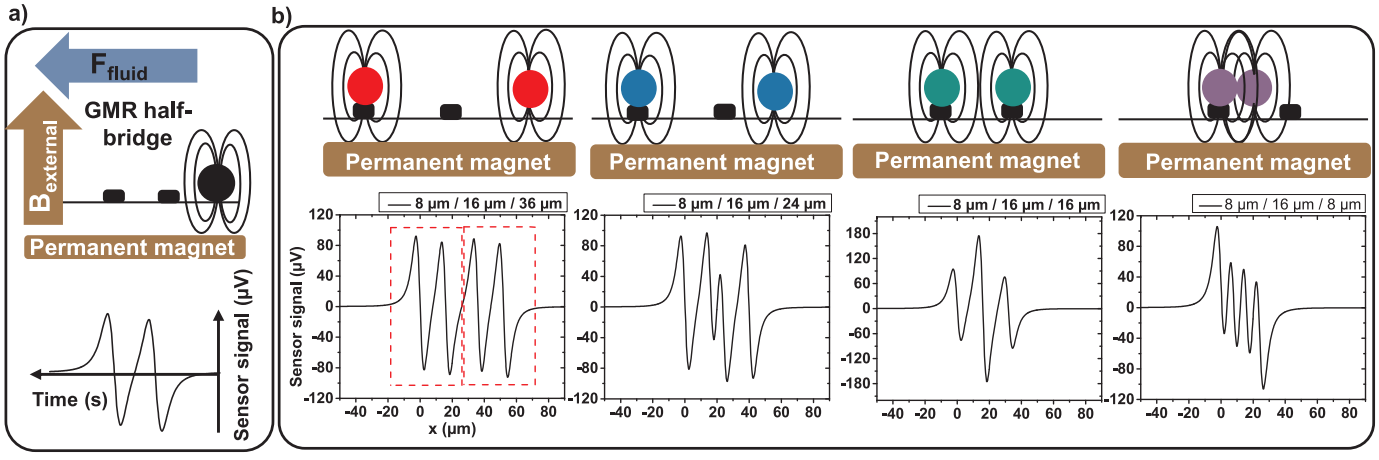

Fig. 1. Detection and workflow schematics of an integrated magnetic flow cytometer with adaptive particle focusing. (a) Detection of magnetic targets in an external magnetic field with a Wheatstone half-bridge comprising two GMR sensors. Single particle information is derived from the characteristic four-peak magnetic fingerprint originating from a single magnetic particle (black) by TOF analysis. (b) Magnetic sensor signals are simulated for two 8 µm particles passing the GMR resistors sequentially. For 16 µm between the GMR elements, signal interference is observed for a particle-particle distance of 8 µm (purple), 16 µm (green), and 24 µm (blue). However, for 36 µm (red), two distinct characteristic four-peak signal patterns (surrounded by dashed boxes) enabling TOF analysis are observed. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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