

ON-CHIP SANDWICH IMMUNOASSAY IN AN INTEGRATED MAGNETO-OPTICAL CMOS MICROSYSTEM

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ABSTRACT

We report on an innovative approach to on-chip detection of single fluorescently-labeled magnetic micro-particles by means of Single Photon Avalanche Diode (SPAD)[1] arrays. Our CMOS-based system enables particle transport through magnetic actuation coils and highly sensitive, high signal-to-noise-ratio detection with integrated SPADs. We demonstrate the quantitative fluorescent detection of fluorescently labeled magnetic particles down to a target antigen concentration of 5 ng/mL. This represents a first step towards a complete lab-on-a-chip system for specific antibody detection using a fluorescent immunoassay.

KEYWORDS: Immunoassay, Magnetic particle, Single Photon Avalanche Diode, SPAD

INTRODUCTION

The use of magnetic micro-particles as the mobile substrate in a bio-assay is motivated by their growing impact on the lab-on-a-chip research area, fueled by their ability of contactless manipulation and a handling that is independent from biological processes [2, 3]. This paper presents the results of on-chip *fluorescent immunoassay detection* on a single particle. We present a hybrid CMOS chip which combines magnetic actuation and optical detection. Decoupling of manipulation and detection principles allows a high sensitivity of the process. We show that integrated SPADs enable discriminating between fluorescently-labeled and non-labeled particles. We also show the capability of differentiating between different concentrations of antibodies bound to the particle surface.

THEORY

Our system consists of a circuit fabricated in a standard four-metal CMOS technology. The characteristics of the chip and its working principle were introduced in [4]. Actuation and detection are decoupled in our system, thus allowing one to achieve higher sensitivity and to avoid interferences. A series of micro-coils creates the magnetic gradient necessary to manipulate the particles, which are transported within a glass capillary positioned on top of the chip (Figure 1). When the particles pass between the microscope light-source and a SPAD placed in the centre of the coils, the photon count decreases as a result of the shadow effect. Lehmann et al. previously reported on the relation between particle size and detector signal [4].

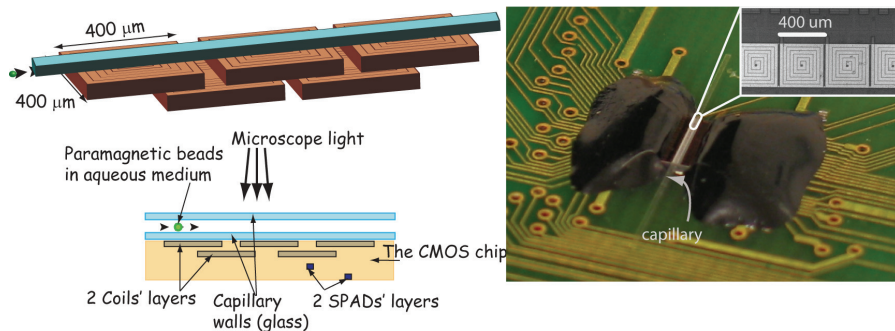


Figure 1. Left: scheme of the CMOS chip (top) and its side view (bottom). Right: picture of the chip, with the glass capillary positioned on the coil array. Insert: zoom of the coil array

For the fluorescent detection we exploit the fact that, when a particle is illuminated from the top, the fluorescent markers create a light-emitting aura around the bead, resulting in an increase of the amount of photons impinging upon the SPAD. A differential measurement allows the extraction of the fluorescent signal (Figure 2).

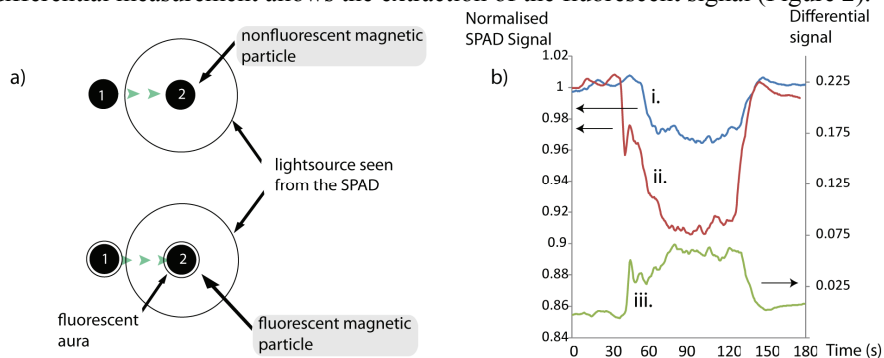


Figure 2. Detection principle. (a) Particles passing as seen from the SPAD. The fluorescent aura causes a higher number of photons impinging on the detector, raising thus the output signal. (b) i. non-fluorescent bead signal; ii. fluorescently labeled bead signal; iii. differential signal obtained by subtracting curve ii from curve i.

EXPERIMENTAL

Firstly, standard off-chip protocols are performed, in order to create an immune-sandwich on streptavidin-coated micro-beads. We use 3 μm diameter beads from Bangs Labs, biotinylated rabbit anti-mouse IgG as capture antibody, mouse IgG as target antigen and Cy3 conjugated anti-mouse IgG as detection antibody[5]. The fluorescent antibodies bind only to the particle surface if the target antigen is present, causing a quantitative fluorescent response of the system directly proportional to the amount of analyte in the studied solution (non-competitive assay). Secondly, the labeled beads are introduced into the glass capillary and are subjected to handling and detection via the CMOS chip. We perform our experiments with different target antigen concentrations.

RESULTS AND DISCUSSION

The differential fluorescent signal shows a clear change, when particles are fluorescently labeled. The response of the system is shown in Figure 3.

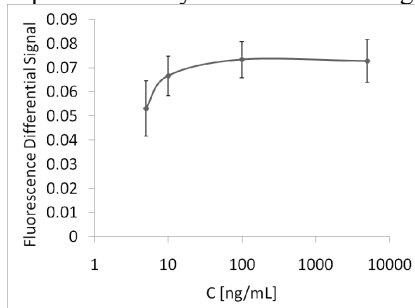


Figure 3. Normalized signal change for different tests.

The graph shows a saturation region when target concentration is higher than 100 ng/mL. The response of the system for concentrations below 5 ng/mL will be studied in the future. The lowest concentration detected in our system is already aligned to the state-of-the-art; however, the extreme sensitivity of the SPADs suggests that even lower target antigen concentrations could be detected.

CONCLUSIONS

We demonstrate the feasibility of the detection and quantification of fluorescent immuno-reactions on a single magnetic particle, via a monolithic magneto-optical CMOS chip. Our results indicate the potential of this approach for innovative, automated techniques for on-chip immunoassays.

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