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Quantification of rolling circle amplified DNA using magnetic nanobeads and a Blu-ray optical pick-up unit

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ABSTRACT

We present the first implementation of a Blu-ray optical pickup unit (OPU) for the high-performance low-cost readout of a homogeneous assay in a multichamber microfluidic disc with a chamber thickness of 600 μm. The assay relies on optical measurements of the dynamics of magnetic nanobeads in an oscillating magnetic field applied along the light propagation direction. The laser light provided by the OPU is transmitted through the sample chamber and reflected back onto the photo detector array of the OPU via a mirror. Spectra of the 2nd harmonic photo detector signal vs. the frequency of the applied magnetic field show a characteristic peak due to freely rotating magnetic nanobeads. Beads bound to ~1 μm coils of DNA formed off-chip by padlock probe recognition and rolling circle amplification show a different dynamics and the intensity of the characteristic peak decreases. We have determined the optimum magnetic bead concentration to 0.1 mg/mL and have measured the response vs. concentration of DNA coils formed from *Escherichia Coli*. We have found a limit of detection of 10 pM and a dynamic range of about two orders of magnitude, which is comparable to the performance obtained using costly and bulky laboratory equipment. The presented device leverages on the advanced but low-cost technology of Blu-ray OPUs to provide a low-cost and high-performance magnetic bead-based readout of homogeneous bioassays. The device is highly flexible and we have demonstrated its use on microfluidic chambers in a disc with a thickness compatible with current optical media mass-production facilities.

1. Introduction

There is a need for low-cost and fast methods at the point of care (POC) level to quantify the presence of bacteria directly from a patient sample. Conventional microbiological methods for bacteria detection are mainly based on culture followed by counting. This is a time-consuming and laborious process. DNA-based techniques have emerged as promising alternatives to microbial detection due to the high sensitivity and specificity of hybridization between the probe and the complementary target sequence (Pechorsky et al., 2009).

Molecular assays based on ligation of padlock probes for target recognition and subsequent rolling-circle-amplification (RCA) (Banér et al., 1998; Dahl et al., 2004) are well-established assays used for rapid, highly sensitive and specific detection of bacteria in molecular diagnostics. The assay specificity allows for high-resolution sequence detection and robust genotyping. In addition, from a technical perspective, it is potentially easier to realize on a chip, as RCA is isothermal and does not require the fast and multiple temperature change needed for amplification by the polymerase chain reaction (PCR).

The RCA product randomly collapses into a coil of DNA, and a site of the coil may be fluorescently tagged facilitating its visual detection as a single bright object of about 1 μm in diameter (Jarvius et al., 2006). For POC applications, this sensing method is not optimal as it requires precise and expensive microscopy-based instruments.

Different detection methods for RCA products based on the use of nanoparticles have lately attracted a considerable interest. Several groups have proposed approaches based on the use of Au nanoparticles alone (He et al., 2014) or combined with electrochemical (Ding et al., 2013) or surface plasmon resonance (Xiang et al., 2013) readouts for the specific and sensitive detection of DNA. The use of magnetic nanoparticles has recently been

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proposed for a simple visual qualitative readout of RCA products (Lin et al., 2013).

In this work, we focus on a sophisticated and sensitive method that exploits the change of the Brownian rotation dynamics of magnetic nanobeads (MNBs) when these attach to DNA coils (Strömberg et al., 2009; Strömberg et al., 2008a, 2008b). Using measurements of the magnetic susceptibility of a suspension of MNBs mixed with the sample containing DNA coils, sensitivities in the pM range have been demonstrated. The method has been successfully demonstrated for the detection of bacterial DNA and spores (Zardán Gómez de la Torre et al., 2012) as well as for studies of drug resistance in *Mycobacterium Tubercolosis* (Engström et al., 2013). In addition to bulky laboratory AC susceptometers, the method has been demonstrated using portable AC susceptometers (Zardán Gomez de la Torre et al., 2011) and magnetoresistive sensors integrated in a microfluidic system (Dalslet et al., 2011; Donolato et al., 2011; Østerberg et al., 2014; Østerberg et al., 2013). Although, these methods have provided significant substantial reductions in cost, size and integratability, they are not easily implemented in a truly low-cost system suited for single use sample analysis.

To overcome these limitations, we have recently developed a novel optomagnetic readout method for measuring the Brownian rotational dynamics of MNBs (Donolato et al., 2014). Using optical laboratory equipment we have demonstrated the detection of RCA products (*Vibrio Cholerae*) using standard cuvettes with a limit of detection (LOD) of about 10 pM, which is comparable to that obtained by the other methods listed above.

Nevertheless, the use of state of the art technologies and high-end optics inevitably limits the real commercialization potential of this technology. Thus, we have focused on the simplification of the electronics and optical components using a Blu-ray optical pickup unit (OPU) as a single excitation and sensing element.

Due to the great potential of low cost, the use of embossed nanostructures in CD and DVD discs as well as the error-correction function of disc drives have attracted interest for biodetection (Yu et al., 2013). Remarkably, a modified DVD OPU combined with an external camera have been recently used for cell counting on a microfluidic disc format (Ramachandraiah et al., 2013) and Blu-ray readers and discs have been proposed for high-density microarray analysis (Arnandis-Chover et al., 2014). To this date, commercial DVD/Blu-ray OPUs have not been used for the readout of homogeneous assays. These hold a great potential for POC applications, as they can be carried out with short incubation times and without washing and surface functionalization of the chip.

In this Article, we show for the first time measurements of the Brownian relaxation dynamics of MNBs using an inexpensive Blu-ray optical pickup unit as the single optical excitation and sensing element and a transparent disc as microfluidic cartridge. The sensing mechanism is based on the dynamic rotation of magnetic nanoparticles, which is now realized for the first time on a format fully compatible with POC requirements. As a proof-of-concept experiment, we use this system to demonstrate molecular detection of DNA coils formed by padlock probe recognition and rolling circle amplification from *Escherichia coli* (*E. coli*). We show that by simply using the laser source from a commercial Blu-ray pickup head ($\lambda = 405$ nm), the Brownian relaxation dynamics of the individual 100 nm MNBs can be detected using the photo detector embedded in the pickup head down to a MNB concentration of 0.02 mg/mL in a microfluidic disc of 600 μm thickness, which is compatible with existing commercial CD/DVD/Blu-ray media production facilities. Using the presented platform we study the dependence on the MNB concentration for the detection of 50 pM DNA coils formed from *E. coli*. Finally, we present the dose-response curve measured on the Blu-ray disc platform for target DNA coil concentrations ranging from 10 pM to 500 pM.

2. Experimental

2.1. Experimental setup

Fig. 1 shows a schematic of the readout system. We used a Sanyo Blu-ray optical pick-up unit from which we removed the last

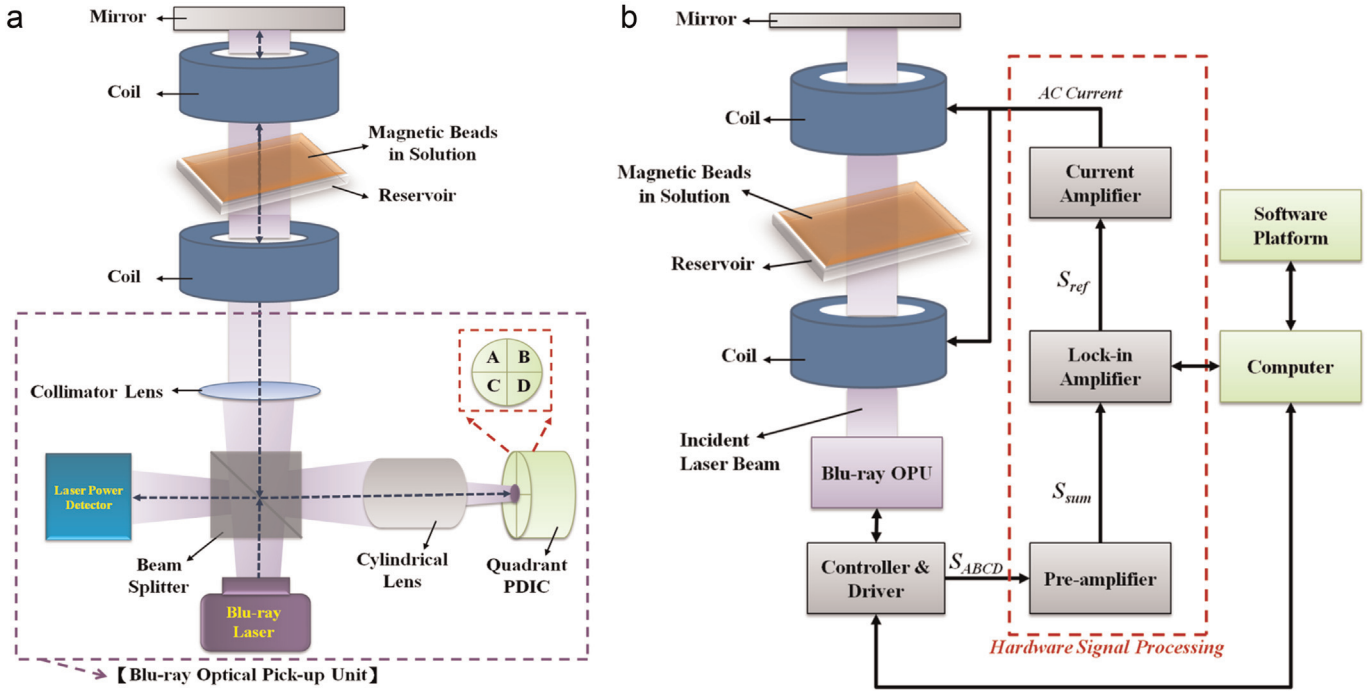

Fig. 1. (a) Optical elements constituting the Blu-ray optical pick-up unit (OPU) and the overall optical system. (b) Schematic of the electronic components used to process the signal from the Blu-ray OPU.