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# Confocal epifluorescence sensor with an arc-shaped aperture for slide-based PCR quantification

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ABSTRACT

The increasing needs of point-of-care diagnostics, quarantine of epidemic pathogens, and prevention of terrorism's bio-attacks have promised the future of portable real-time quantitative polymerase chain reaction (qPCR) sensors. This work aims at developing a highly sensitive and low-cost light emitting diode (LED)-based epifluorescence sensor module for qPCR sensor development and relevant bioassay applications. Inspired by the light stop design and dark-field detection of microscopes, this paper first reports a compact confocal LED epifluorescence sensor using a light stop with an arc-shaped aperture for enhancing the flexibility of quick DNA and PCR detection. The sensor features the advantages of the dichroic mirror-free and confocal (shared-focus) characteristics, which benefits size reduction and minimal optics used. It also allows extension to integrate with *in situ* real-time PCR thermal cycling since the sample slide is placed apart from the epi-sensing module. The epifluorescence sensor can detect as low as sub-ng/μL standard DNA and $10^1$ copies of *Salmonella typhimurium InvA* gene sequences (cloned in *E. coli* and after 30-cycle PCR) with SYBR® Green I from non-purified culture samples, having highly sensitive and specific signal responses comparable with that of a commercial qPCR instrument.

## 1. Introduction

Specific amplification of a target DNA exponentially with primers and three-temperature thermal cycling, polymerase chain reaction (PCR), the Nobel prize-recognized biotechnique (Saiki et al., 1988), has become a common laboratorial method. It has been extensively applied for gene sequencing, nucleic acid detection, molecular cloning, and a wide variety of bio-related applications in the past two decades (Rahman et al., 2013). Eliminating tedious post-PCR gel electrophoresis analysis, the advent of the real-time quantitative PCR (qPCR) instrument (Wittwer et al., 1997) has further boosted the importance of PCR in analytical sciences. Recent increasing demands of point-of-care diagnostics (Liu et al., 2009; Song et al., 2014), quarantine of epidemic pathogens (Baca et al., 2015; Sun et al., 2012), and prevention of terrorism's bio-attacks (Franz et al., 1997; Shi et al., 2015) have driven new desires for portable biosensors capable of on-site, rapid DNA analysis. Emerging approaches, such as electrochemical PCR (Defever et al., 2011; Lee et al., 2003) and isothermal nucleic acid amplification (Asiello and Baeumner, 2011; Notomi et al., 2000), have been investigated for this propose. On the other hand, miniaturization and improvement of a fluorescent qPCR instrument (Ahrberg et al., 2016; House et al., 2010; Koo et al., 2013; Wang et al., 2009) provide the most straightforward and reliable solutions to attain the goal. For examples, the development of chip-based PCR (Park et al., 2016; Qin et al., 2016) enables epi-mode detection and pipette-free operation to simplify the sensing process. In addition, smartphone cameras have been demonstrated capable of quantification of faint fluorescence (Kong et al., 2017; McCracken and Yoon, 2016; Priye et al., 2016) and semi-quantification of droplet digital PCR (Cao et al., 2017; Hindson et al., 2011; Tanaka et al., 2015) for rapid on-site diagnostics. Considering this trend, we aim to construct a novel sensitive and low-cost fluorescent sensor module for portable PCR sensor development. The sensor is designed with epi-fluorescent detection mode (*i.e.*, excitation source and emission detector are placed at the same side against the sample, see Fig. 1) to reduce the size and to prevent optical interference when integrated with a PCR thermal cycler.

Fluorescent qPCR detection can be carried out with two different kinds of reporters – sequence-specific molecular beacon (e.g., TaqMan® probe) (Alasaad et al., 2011; Carisse et al., 2009) and universal dsDNA-staining dye (e.g., SYBR® Green I dye) (Pfaffl, 2001; Ponchel et al., 2003). Considering the generalized application of a portable qPCR biosensor, we choose SYBR® Green I dye (excitation maximum at 497 nm; emission maximum at 520 nm after binding to double-stranded DNA) as the reporter for sensor development. The dye

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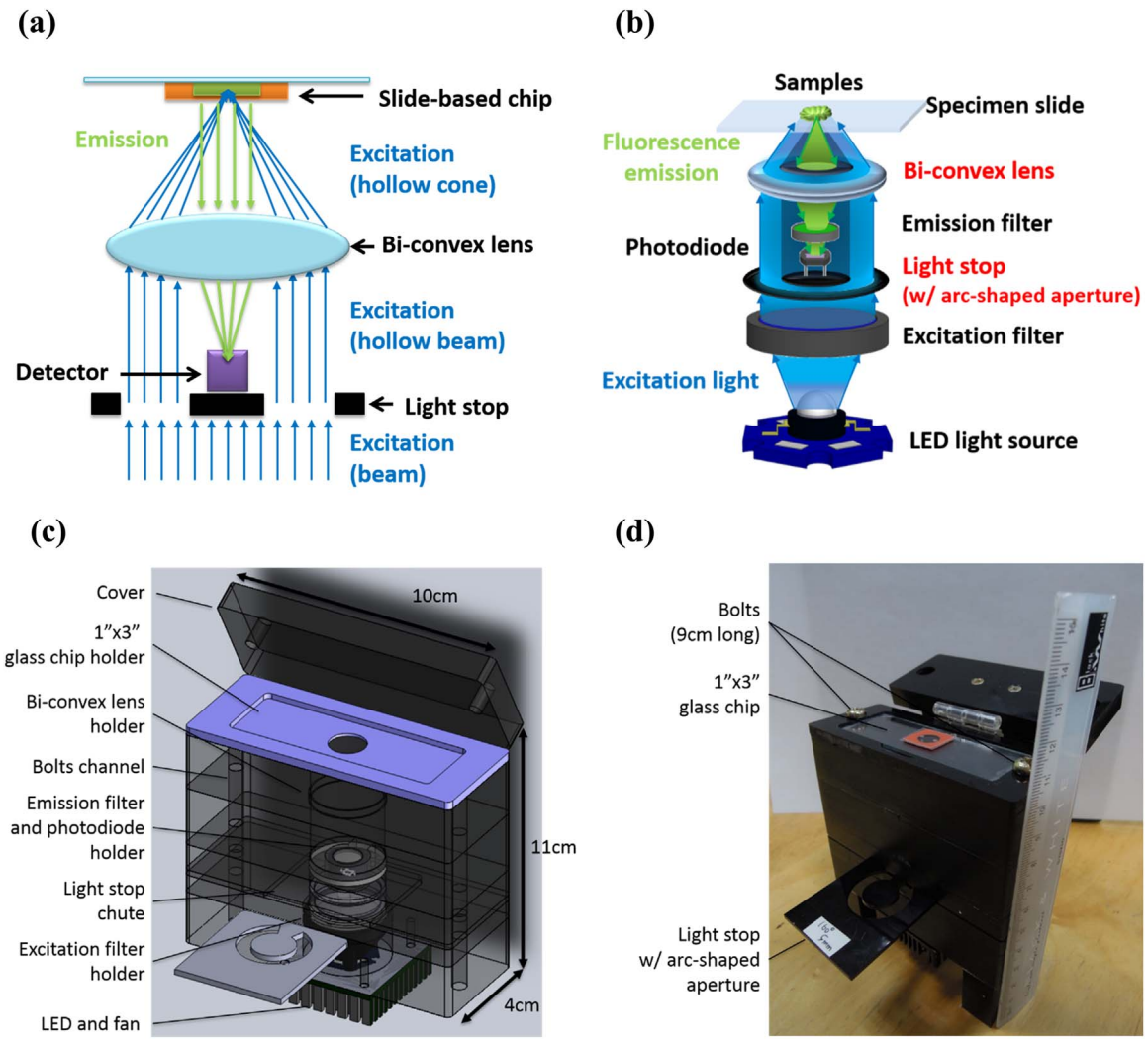


**Fig. 1.** (a) An illustration of the light paths for explaining the confocal characteristics and formation of the hollow cone light for excitation using the light stop with arc-shaped apertures. (b) The scheme of the present LED confocal epifluorescence sensor. (c) The CAD model of the blocks used for mounting the optical elements. (d) The appearance of the sensor assembly. All blocks are settled and fixed by two bolts (about 9 cm), except for the glass chip and light stop are exchangeable.

can detect as low as 20 pg DNA (Nath et al., 2000; Zipper et al., 2004). The molecular structure, excitation and emission spectra, and sensing mechanism of SYBR® Green I are given in Fig. S1 in Supplementary materials. Ordinary qPCR machines use high-pressure mercury or xenon arc lamps (Beer et al., 2007; Lee et al., 2004; Zhang et al., 2011) to excite the reporter dye, while some other high-throughput or small-spot detection instruments utilize scanning lasers (Chang et al., 2013) as excitation sources. In this work, we choose a high-luminous-flux blue LED (with a peak intensity at 470 nm) to trigger the dye's emission for its advantages of small size, low cost, low energy consumption, quick response, and near single-wavelength light source (Buah-Bassuah et al., 2008). The reported LED sensor configurations for fluorescent PCR detection can be classified into straight, oblique, vertical and reflective types (see Fig. S2 in Supplementary materials), respectively, according to how the excitation light shines on the sample and the emission light is collected by the detector. As can be seen in Table S1 in Supplementary materials, a number of published papers have demonstrated the applicability of LED in PCR quantitation, and both oblique and reflective types of sensor configuration can meet the epi-fluorescence detection requirement. To further reduce the sensor's size and cost, we choose a silicon photodiode (George et al., 2013) for detecting the reporter dye's emission. Although Table S1 indicates that the LED/photodiode-based qPCR system has a potential to detect as low as 3 copies of target gene with the aids of sophisticated microfluidic PCR chip and optical designs (Belgrader et al., 2003), it is also observed that most photodiode-based sensors can only detect $10^6$–$10^9$ copies of target gene (Mondal and Venkataraman, 2005), which seems less promising than others. This means how to greatly improve the sensitivity of LED/photodiode-based PCR detection to a great extent remains an important issue. Besides, there also needs workable ideas to shorten the LED-to-photodiode distance and minimize the optics. To cope with these issues, we employ the dark-field detection with a light stop strategy used in microscopy to improve the sensitivity of LED/photodiode-based PCR detection and to minimize the sensor size.

Dark-field detection is a well-known microscopy approach for increasing the image contrast, and it typically uses a light stop and a condenser (Cardioid or Abbe type) (Matsuzaki et al., 2004) to form a hollow cone of light with oblique incidence for preventing the background interference from the reflected excitation light, as illustrated in Fig. S3 in Supplementary materials. This suggests that the light stop design plays a determinant role in getting high-resolution microscope images as well as sensitive optical signals with high signal-to-background (S/B) and signal-to-noise (S/N) ratios (Lin et al., 2009). In addition, it inspires us to use a light stop with an arc-shaped aperture to replace a dichromic mirror (beam splitter) for separating the paths of excitation and emission lights and to achieve sensitive dark-field, epi-mode fluorescence detection, which has not been applied to LED fluorescent sensors before. Accordingly, the idea of a confocal epifluor-