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Integrated OLED as excitation light source in fluorescent lateral flow immunoassays

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ABSTRACT

The integration of organic light emitting diodes (OLEDs) as excitation light sources for quantum dot-based fluorescent lateral flow immunoassay systems (LFIA) was investigated. This approach has the potential to deliver a sensitive visible detection scheme for low-cost, disposable lab-on-chip point-of-care (POC) diagnosis system. Thin film phosphorescent green OLEDs fabricated on plastic substrates were integrated on-chip to excite the test line of a quantum dot-based LFIA (QD-LFIA). OLEDs were fabricated by sequential deposition of organic thin films (total of ~100 nm) onto ITO-coated PET substrates. CdSe/ZnS QDs emitting at 655 nm and Au nanoparticles (NP – 10 nm size) conjugated antibodies were used for the fluorescence QD-LFIA and conventional reflection-mode Au NP-LFIA, respectively. Thin plastic color light filters were integrated for filtering the excitation light source and, thereby, increasing the contrast of the emitted light for optimized visual detection. Integration of the OLED and color filters with the analytical membrane was achieved using adhesive techniques facilitated by the planar nature of the layers, which suggests possible large scale manufacturing using roll-to-roll processing. Gray scale analysis from digital images captured with a digital camera was used to quantify the visual sensitivity. The signal intensity, signal-to-noise ratio (SNR) and the limit of detection (LOD) of OLED integrated QD-LFIAs were compared to Au NP LFIAs. OLED QD-LFIA exhibited superior performance in all signal aspects: 7–8 × higher signal intensity and SNR, and a 7 × lower LOD of 3 nM (measured at $S/N=3$). These results demonstrate the potential of OLED-integrated in LFIA devices for obtaining sensitive, fast and low-cost POC diagnostics.

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1. Introduction

Lab-on-chip (LOC) concepts have provided a successful path for the development of low-cost and disposable point of care diagnostic (POC) devices. LOC focuses on ways to miniaturize laboratory scale equipment to perform diagnosis on a small scale, thereby making the system cheap and portable. Microfluidics is the key underlying technology that enables miniaturizing of many LOC diagnostic devices (Haeberle and Zengerle, 2007; Roman and Kennedy, 2007; Yager et al., 2006; Yi et al., 2006). Paper-based devices are currently being investigated (Steckl, 2013; Tobjörk and Österbacka, 2011) for a variety of applications such as electronics, displays and sensors because of the inherent low cost of the material and of roll-to-roll manufacturing. Microfluidic devices that use capillary transport in paper represent a very attractive and surprisingly versatile path for low cost LOC devices (Fu et al., 2011; Martinez et al., 2009; Parolo and Merkoçi, 2013; Yager et al., 2006). Immunochromatographic assays, also known as lateral flow immunoassays (LFIA), use capillary wicking for the transport of analytes to the detection zone where the immunoreaction takes place (Ngom et al., 2010; Posthuma-Trumpie et al., 2009). The inherent capillary pump action integrated in the diagnostic device removes a major drawback of polymer (such as PDMS, PMMA or PC) based microfluidics devices, which normally require external pumps for fluidic transport and manipulation (Yetisen et al., 2013). In addition to their low cost, LFIA devices operate rapidly (Yetisen et al., 2013), with the detection of the analyte performed in a few minutes. The outcome of the immunoreaction can give a simple qualitative ("yes/no") answer in nature (Posthuma-Trumpie et al., 2009). Many such assays commonly use colloidal gold (Hirsch et al., 2003; Kolosova et al., 2007; Krska and Molinelli, 2009; Kusano et al., 2007; Ngom et al., 2010; Posthuma-Trumpie et al., 2009; Verheijen et al., 1998) nanoparticles (Au NP), which when accumulated in the test line region of the LFIA appear reddish in color and thereby giving a qualitative visual readout for the presence of analyte. A widely utilized commercial Au NP-LFIA is the pregnancy test strip, which detects human chronic gonadotropin (hCG) hormones in urine specimens (Tanaka et al., 2006). Multiple

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commercial products use Au NPs as the label for hCG pregnancy tests.

The use of fluorescent particles in these assays (Bamrungsap et al., 2014; Corstjens et al., 2008; Gui et al., 2014; Xia et al., 2009) has recently gained considerable interest due to the resulting high LFIA sensitivity (Xie et al., 2014). Quantum dot (QD) fluorescent particles, owing to their high photoluminescence properties (Lu et al., 2003), as well as colloidal water soluble synthesis methods (He et al., 2007), are being increasingly explored in medical applications (Cui et al., 2008; Han et al., 2001; Li et al., 2010b; Montón et al., 2012; Taranova et al., 2015; Zhang et al., 2009). Recently, QDs have received attention for incorporation into LFIA devices. Syphilis detection using QD-LFIA reported by Yang et al. (2010) has shown 10× improvement in visual limit-of-detection (LOD) by using QDs over conventional Au NP-based system. Similar improvement of visual sensitivity, were reported by Li et al. (2010a) in detecting human ceruloplasmin. Quantitative measurements were performed (Li et al., 2010a) with the QD-LFIA using an external reader unit. These results show the potential of fluorescent particles as visual indicators in LFIA devices. However, these LFIA systems also require separate readers with light sources and detectors, usually benchtop units that are not easily portable and thus harder to use in POC applications.

Organic light emitting diodes (OLED) consist of a series of thin films of various organic materials deposited on substrates, resulting in devices that emit and detect light when biased (Tang and VanSlyke, 1987). While OLEDs are usually formed on glass substrates, the fact that the thin film deposition takes place at relatively low temperatures makes it possible to fabricate them on plastic films or paper substrates (Purandare et al., 2014; Zocco et al., 2014). This facilitates integration with LFIAs for realizing LOC applications (Williams et al., 2014) for medical diagnostics. OLEDs have several advantages compared to their inorganic counterparts (LED), including physical flexibility and large area fabrication capability (Williams et al., 2014). Fluorescence detection in plastic microfluidic chips using separate OLED excitation has been reported by Pais et al. (2008) to exhibit sensitive detection (100 nM). However, a system that integrates OLED with paper microfluidic devices has not been realized yet. Such an integrated system would take the advantage of both paper-based diagnostics as well and the potential of organic optoelectronic devices.

In this manuscript, we explore the integration of OLEDs as the excitation source in QD-LFIA devices for high sensitivity, visual observation-based qualitative LFIA diagnosis. Red emitting (655 nm) QDs were used as fluorophores in the LFIA. Though these QDs have maximum absorption in the UV/blue region, the higher efficiency and brightness of green OLEDs compared to blue OLEDs, led us to choose the former. To enhance the visual signal from the QDs in the test line of the LFIA, two color filters were also incorporated in the device. Fig. 1 shows an overall sketch of the integration concept.

The QD-LFIA was compared with conventional Au NP-based LFIA operated under similar conditions in order to ascertain improvements in contrast and LOD. A high sensitivity system will improve the LOD in several LFIA systems that provide qualitative (i.e. yes/no) analysis, such as home pregnancy test kits and flu kits.

2. Materials and methods

2.1. OLED fabrication

A phosphorescence based green emitting OLED stack, ITO/NPB/CBP:Ir/BCP/ALQ3/LiF/Al, was chosen due to its inherent high efficiency and brightness, as demonstrated by the Forrest group (Adachi et al., 2001). Energy levels of the organic layers stack are shown in Fig. 2a.

OLEDs were fabricated on ITO-coated PET sheets (5 mil) having a sheet resistance of 60 Ω/square (Sigma Aldrich). To facilitate ease of fabrication, PET sheets were attached to rigid glass substrates before processing. The fabrication process starts by lithographically patterning the ITO to produce 4 mm wide strips. After ITO patterning, the surface was cleaned using O_2 plasma (250 W power) for 2 min. The substrates were then transferred to a high vacuum deposition system and the organic layers were sequentially deposited through a shadow mask at an operating pressure of 5×10^{-7} Torr. The thickness of the total organic stack was ~100 nm. Next, the substrates were very briefly removed to load the anode mask. Lithium fluoride (LiF) and aluminum were then deposited (total of ~40 nm) in the vacuum system, forming the anode electrode in devices with an active area of 4 mm × 4 mm. After the OLED fabrication was completed, the PET sheet was removed from the glass substrate in order to integrate the OLED with the LFIA. Each OLED substrate measured 15 mm × 15 mm, as shown in Fig. 2b. It is important to note that in these bottom-emitting OLEDs, the emission that is utilized in the overall device propagates through ITO layer and the PET sheet.

OLED current–voltage (*I–V*) characteristics were obtained with

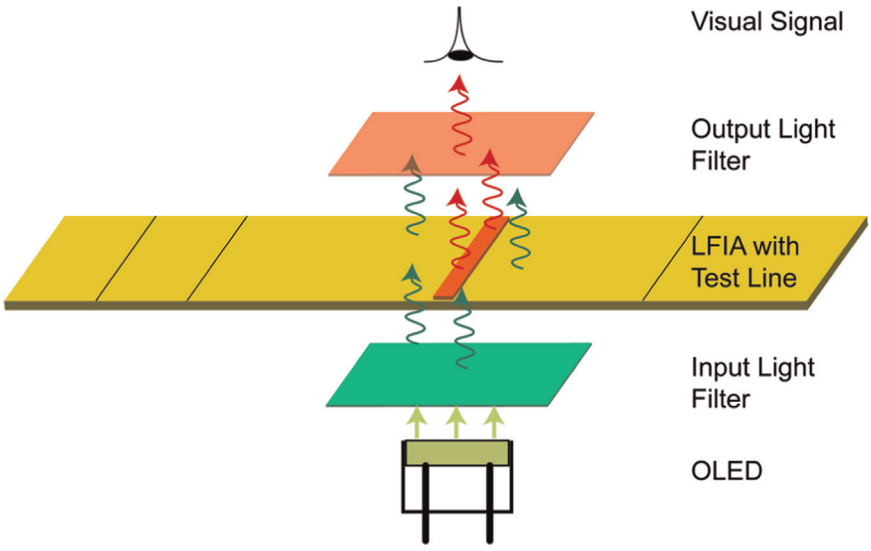

Fig. 1. Schematic of OLED/LFIA integration approach.

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