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PAPER

Integrated NFC power source for zero on-board power in fluorescent paper-based lateral flow immunoassays

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Abstract

A lab-on-chip vehicle was explored combining RF near field communication (NFC) harvested power and light sources for paper-based lateral flow immunoassay systems (LFIA) with quantum dots (QD) as fluorophores. Such a vehicle has potential applications in point-of-care systems requiring high sensitivity while also being low-cost, disposable and easy to use. Micro-LEDs which provided the excitation source for the test line of a QD-LFIA were surface mounted on plastic substrates using a printed hybrid electronics approach for the fabrication of power harvesting NFC antenna, chip assembly and electronics integration with the LFIA strip. The LFIA is a rotavirus assay kit with Au nanoparticles into which QDs emitting at 655 nm were also incorporated. A digital camera was used for detecting the fluorescence from QDs and the reflected signal from Au NPs. The signals were compared using grayscale analysis. The NFC-powered LED light source integrated with the QD-LFIA demonstrated ~9× higher sensitivity compared to conventional Au-NP based assays. Such an integrated system can be potentially mass manufactured using roll-to-roll processing making the device cost effective, as well as having high sensitivity.

1. Introduction

Lab-on-chip (LOC) technology is focused on miniaturizing laboratory processes to the chip level, thus enabling portable and cost effective diagnostic systems. This also enables the use of reduced sample volume, which is critical in several applications [1, 2]. Microfluidics platforms have been developed to handle fluids of such low volume and typically form the core of LOC systems [3–6]. Though plastic (PDMS)-based soft lithography is widely utilized in microfluidics [7], paper-based devices are also very attractive, owing to their low cost and ability for capillary transport [8]. Lateral flow immunoassays (LFIA) are one such example of successful application of paper-based devices that utilize immunoreactions of antigen and antibody (Ab) molecules at detection zones, known as test and control lines [9, 10]. Transport of the analytes in the sample solution is by the capillary action of paper, which makes such devices attractive over PDMS-based devices that typically require external pumps to drive liquids [8]. The outcome of such devices are typically qualitative (yes/no), which is inferred visually from the change of color when colloidal gold nanoparticles (Au-NP) collect on the test line [11–13]. One such widely utilized LFIA in the commercial world is the pregnancy test that detects the presence of human chronic gonadotropin (hCG) hormones in urine specimens [14]. Applications of paper-based devices in electronic displays and sensors have also been pursued by several groups [15, 16]. Such devices also have an inherent advantage of being amenable for large volume and low unit cost using roll-to-roll manufacturing.

Given all the desirable characteristics of LFIAs, they still fall behind on sensitivity and limit-of-detection (LOD) in comparison to enzyme-linked immunosorbent assay (ELISA) [17]. A possible approach to improve LFIA sensitivity is the use of fluorescent particles over conventional Au nanoparticles (NP) as the colorimetric indicator in the assays [18–21]. Specifically, quantum dots (QD) as fluorescent particles have gained considerable interest in medical applications [22–24]. Some of their desirable characteristics over other assay particles are high photoluminescence and simple synthesis using colloidal water soluble methods [25, 26]. The incorporation of QDs in LFIA devices has been reported by several groups. Particularly noteworthy for the application targeted in this article are those of Yang *et al* [27] who have shown a 10× increase in visual signal and Gui *et al* [20] who used CCD sensors to integrate the photo signal for best sensitivity and LOD. Several groups investigated making the assay more quantitative, along with being more sensitive, by using QDs in conjunction with external reader systems [24, 28, 29]. Major limitations in the work of these groups for point-of-care (POC) applications were the requirements for external readers, light sources and power supplies. We have previously reported [30] one solution to this limitation by integrating organic light emitting diodes (OLEDs) as a light source and successfully produced similar improvements in sensitivity. However, there remains the need to integrate the power source into the device as well.

Thin film batteries are possible candidates for this application as they can be flexible, thin and biodegradable [31, 32]. However, an approach having a zero on-board power and harvesting energy from ubiquitous sources may be more desirable [33]. RF harvesting [34] on board is a possibility, however a limitation can be the availability of the source of RF power. The incorporation of smartphones into everyday existence has made their image collection/transmission and computation available for much more than communication. Smartphones have increasingly gained substantial interest as adjunct platforms in diagnostic applications [35–37]. Shen *et al* [38] used a smartphone's camera and CPU for detection and signal processing applied to colorimetric assays. Many modern smartphones possess near field communications (NFC) which uses RF technology for connecting devices. Lee *et al* [39] used this technology for communicating data to/from the diagnostic device. In our approach we focus on power harvesting from NFC-equipped smartphones. NFC harvesting antennas can be hybrid manufactured on flexible substrates, which implies that they can ultimately be mass manufactured using large scale roll-to-roll technology, potentially being cheaper than thin film battery options. Of course, in addition to serving as the power source, NFC may also be used for communication between the diagnostic device and smartphone for processing and cloud connectivity.

In this manuscript we explore NFC-powered green LEDs as light sources in QD-based LFIAs. While OLEDs have the capability for large emission area that can be matched to LFIA geometries, they currently still have emission and lifetime limitations compared to inorganic point-source LEDs. Suitable optical filters have been selected for maximum sensitivity. A conventional CCD digital camera was used for detection, providing the ability to integrate the fluorescent signal for the best sensitivity. Figure 1 shows an overall sketch of the LFIA operation, illustrating the integration of the LFIA with the antenna, LEDs and optical filters.

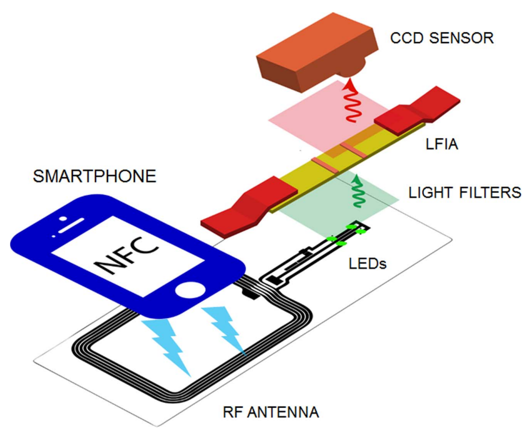

Figure 1. Schematic showing NFC power harvesting and integration with LEDs, optical filters and LFIA.

For the LFIA, a conventional Au-NP based commercial test kit was used. QDs introduced into the test kit with the sample solution are captured on the test line along with Au NPs. The presence of both types of detector particle in the same test line results in an easier and fair comparison. Since the major focus is to obtain improved sensitivity, the lower concentration region is of more interest. This makes interference of one detector type over the other negligible. To quantify the signal, a grayscale plot of the image was taken and the contrast was calculated.

2. NFC LED chip fabrication

The electronic backbone circuitry was fabricated using a hybrid electronics manufacturing approach for reduced device cost, waste and footprint. Copper-coated PET foil (Prinel Ltd, Finland) with a thickness of 50 μm was patterned by direct printing of an etching paste to create the antenna structure and electronic circuit design. Four bare-die green LEDs (ES-CEGHV15B, Epistar Corp., China, thickness 100 μm) and electronic components responsible for the power transfer between mobile phone and the LED chip were assembled onto the flexible circuit using anisotropic conductive adhesive (ACA) bonding on a custom made roll-to-roll hybrid assembly machine at VTT's printed and hybrid electronics pilot manufacturing environment (Datakon EVO 2200, Austria, equipped with custom roll-to-roll feeder unit). The electronic components used for harvesting the RF energy from the antenna structure are a RFID chip responsible for phone-to-device communication (Mifare Ultralight MF01CU2101DUD, 120 μm thickness), and two capacitors for impedance matching with energy harvesting antenna and stabilization of the LED illumination (2 × 50 pF capacitors, 100 μm thickness). All

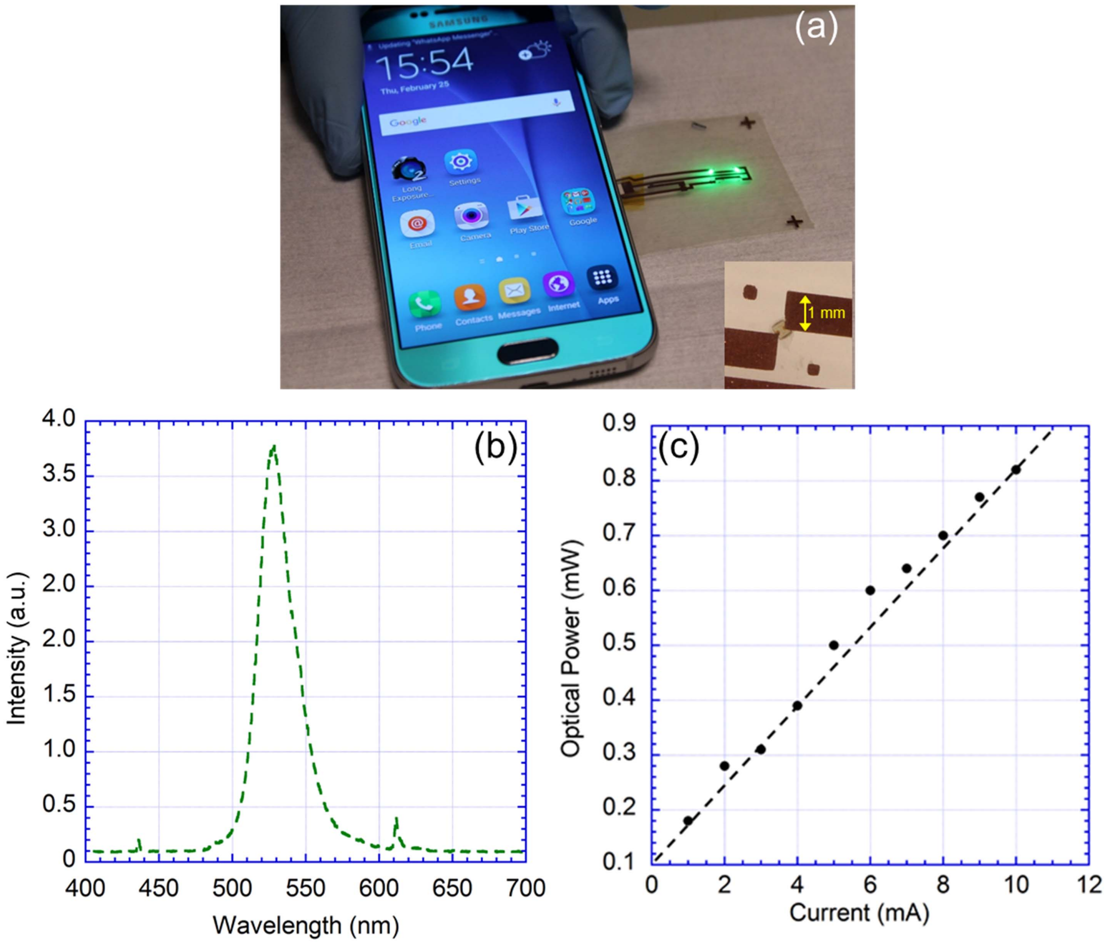

Figure 2. NFC power harvesting for LED operation: (a) demonstration of smartphone powering LEDs (insert—photo showing LEDs surface mounted on plastic sheet); (b) emission spectrum of the green LED; (c) LED output optical power versus input current, separately measured.

components are bare-die silicon chips to reduce the device thickness to a total thickness of 170 μm including the PET substrate.

Figure 2(a) illustrates the powering principle of the flexible LED chip using the NFC module of a smartphone. The LEDs were separately characterized electrically and optically using an external DC bias source. Figure 2(b) shows the spectral characteristics of the green LEDs used as measured using Ocean Optics SD 2000 spectrometer. Optical power output shown in figure 2(c) was measured (Newport Optical 1918-C) against various input currents (HP-6634B DC power source). This plot was used to calculate a power output value of 80 μW (per LED) when powered with 6–7 mW using NFC from a smartphone.

3. LFIA assay fabrication

The LFIA test kits were provided by Meridian Bioscience Inc. (Cincinnati, USA) and designed for detection of rotavirus. The operating principle of the LFIA is illustrated in figure 3. The test strip contains mouse anti-rotavirus conjugated to Au NPs as detector particles in the conjugate pad. The kit also contains a nitrocellulose analytical membrane with capture antibodies (rabbit anti-rotavirus) embedded in the test line that bind to an epitope of the rotavirus molecule. A control line containing goat anti-mouse Ab is present to validate the test, by capturing the conjugate mouse Abs even in the absence of rotavirus analyte. QD 655 (Life Technologies) QDs conjugated to donkey anti-mouse Ab that target the mouse antibodies in the conjugate pad were introduced with the sample solution. An antibody complex is formed as shown in figure 3(c) which then migrates toward the test line and is captured in the presence of analyte. Hence, both QDs and Au-NPs are present in the test and control lines, as can be seen in figures 3(a), (b).

The QDs possess wide absorption spectra in the UV-green region, but a very narrow emission line at 655 nm. This large Stokes shift makes it easy to choose simple colored plastic light filters to eliminate the excitation light signal. For the output light filter, a medium red (Rosco Labs) filter was used. Though the LED has a fairly narrow emission spectrum, an input light filter was used to ensure that there is no competing red spectral component from the excitation source. A Chroma green light filter (Rosco Labs) was used for this

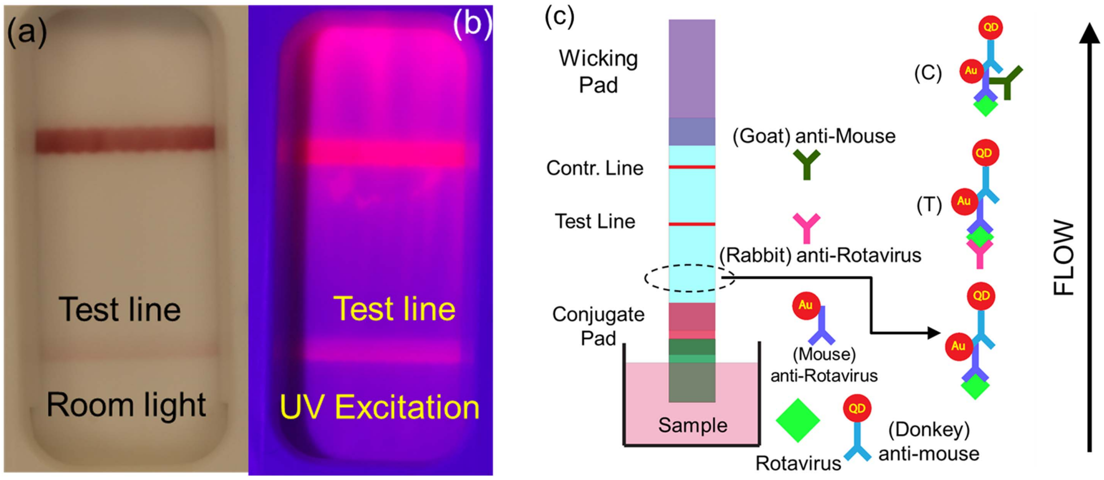

Figure 3. LFIA working principle: (a) captured Au-NPs reflection under room light; (b) captured QD particles fluorescence excited under UV light; (c) immunochemistry of the LFIA showing capture of both Au-NP and QD at test and control lines.

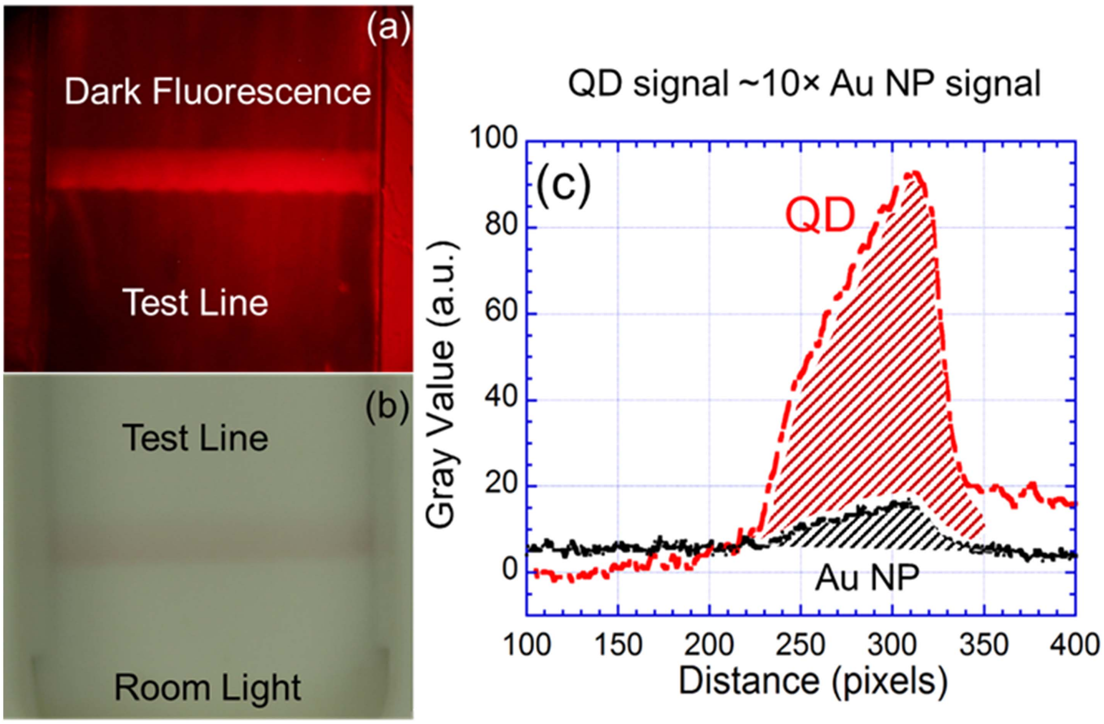

Figure 4. LFIA capture test line results: (a) fluorescence of QDs excited using NFC powered LEDs; (b) room light reflectance of Au NPs; (c) gray value contrast comparison between QD and Au-NP as baseline.

purpose. The spectral characteristics of the QDs and light filters have also been discussed in detail elsewhere [30].

4. Results & discussion

The rotavirus test kit was run with a standard positive solution at a low concentration volume of 10 μl to yield a faint looking test line. Such a concentration would represent the LOD of this commercial test kit using Au-NP and considered as the baseline. 3 μl of QD solution at 1 μM concentration was added along with the negative control solution (100 μl) and the test was run. After subject to a uniform drying process, the membrane with captured test lines (QD & Au) was separated from the test kit and integrated with the light source. The input and output light filters were attached to either side of the analytical membrane using adhesive. The setup consisted of NFC-powered LED as the light source and a spacer. The spacer was a 5 mm thick cardboard with a circular opening. A digital CCD camera was used as the sensor. The integration capability of the CCD by adjusting the shutter speed settings was used to obtain the maximum signal for the

QD-based LFIA. This helped to mitigate the loss in intensity due to spacers.

Figures 4(a), (b) show the test line contrast under optimum conditions for each case: room light conditions for Au NP case, dark fluorescence for QD case. As can be seen from the images, the QDs excited with light powered by the NFC power source exhibit superior performance over Au NPs of the same concentration.

The image was converted to its equivalent grayscale image using ImageJ [40] software and a line contrast plot was measured. For the best results, red channel conversion was selected for QD devices. As can be seen in figure 4(c) the contrast plot is consistent with the images. The signal intensity of the test line was quantified by calculating the area under each curve between 200–350 pixel distance. A ~10× increase in intensity for QD devices compared to conventional Au-NP based diagnostic kit was observed.

It should be noted that the signal contrast of the test line is still limited by the amount of non-specific binding around the test line. Hence, in an effort to bring it closer to an ideal case, a wash run was also performed to remove the non-bound conjugate. However, in the eventual optimized case (i.e. no non-specific binding), the contrast can be amplified by integration to the saturation level and thereby even higher sensitivity can be achieved.

5. Summary

In summary, a lab-on-chip vehicle powered with NFC RF power from smartphones was demonstrated. Fluorescent QD-based paper LFIAs were excited with NFC-powered LEDs fabricated by hybrid manufacturing processes on plastic substrates. A CCD camera was used as detector that integrated the signal to provide maximum sensitivity. In the future the separate camera may be replaced with the camera of the smartphone that is also acting as a power source. The devices achieved nearly 10× increase in sensitivity in comparison to conventional commercially optimized Au-NP based LFIAs.

Such a high sensitivity device was achieved with a minimal increase in device complexity by using a printed and hybrid electronics approach for fabricating a low-cost, disposable LoC device. The added costs for the printed electronics backbone (plastic substrate, conductive ink, antenna, RFID chip, micro-LEDs) is currently in the range of tens of cents (<1 $US) for mid-volume manufacturing (<1 million devices/year) and has the potential for further cost decrease at higher volumes and continued progress in printed electronics research. These results indicate the potential path for future work, such as integrating a photodiode with the device to obtain an electrical output that can then be transferred to the smartphone via the same NFC technology. The optoelectronic part may also be replaced with its organic counterparts (OLEDs and OPDs) for larger area detection, as well as biodegradability and cost effectiveness. Such applications can also bring quantitative detection along with increased sensitivity that are some of the desirable characteristics of Lab-on-Chip diagnostic devices.

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