Integrated multifunctional fluorescence biosensor based on OLED technology

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Abstract—This paper describes a fluorescence biosensor based on a microfluidic device where an organic LED is used as light emitting source. The device was able to detect fluorescence from a 0.012 µl volume chamber containing 3 ng of donkey anti-sheep IgG (Immunoglobulin G) conjugates tagged with Alexafluor 488 fluorophores. The minimum detectable concentration of the antibody was identified at an enzyme concentration of 200 µg/mL. This result shows that organic LED sources can be used as emitter/photodetector pairs to test biological samples. Based on our results, a multipurpose, integrated biological sensor that can identify and measure the concentration of fluorophore substances is discussed.

I. INTRODUCTION

There exist a variety of sensing schemes like electrochemical, optical absorption, and interferometric sensing [1]. However, fluorescence sensing remains the most widely used technology in chemical and bio-medical sensing, substance identification, food processing, mainly because fluorescence detection offers higher sensitivity and accuracy in comparison to other spectroscopic techniques. The shortcomings of traditional instruments are the bulk and efficiency, the need for precise calibration procedures, the need to compensate in different environments, and the complexity of analyzing the fluorescence data in real-time. In heterogeneous surroundings, contaminated by various sources of noise, the analysis of the acquired spectra (original signal) is practically impossible without resorting to sophisticated data processing tools or substance-specific information. This has led to the development of ad-hoc acquisition techniques that are specific to given problems and cannot be generalized. There are several current techniques to measure fluorescence [2], most of which are application specific. Hence a need for general-purpose, integrated solutions that allow the study of the physical and chemical properties of various substances, and the identification of these substances with their concentrations in various contexts is demanded. Additionally, miniaturization and integration of biosensor platforms is appealing due to smaller reaction volumes, larger numbers of detection sites and integration of various functionalities.

LEDs are compact, stable and energy-efficient light sources. A multi-band LED-based fluorescence sensor was proposed by Boukadoum et al. [3]. The system consisted of 8 LEDs that successively served as excitation sources and photo detectors.

The acquired data was amplified and filtered and then categorized by a neural network. In order to increase the system’s immunity to ambient noise during acquisition of the fluorescence signal, a synchronous modulation-demodulation scheme was used. The device was more cost effective and less bulky in comparison to monochromator-based sensors. The main drawback of this inorganic LED-based sensor is its integration complexity, with 8 LEDs successively serving as excitation sources and photodetectors, and the need to use different LED process technologies in order to cover the whole light spectrum from UV to IR. In order to overcome these pitfalls we propose here to use organic LEDs and photo detectors. Due to ease in fabrication, low cost and an emission which can cover the whole UV-visible spectrum, organic materials appear to be an alternative and better solution in fabricating this type of device. The block diagram of a single unit for the white OLED (WOLED) based biosensor is shown in Fig. 1.

![Block diagram of one unit of WOLED based biosensor](image)

In order to investigate the fabrication of a fully integrated bio-sensors we have to couple the fluorescence detector with a microfluidic device. Microfluidic devices have recently attracted remarkable attention due to their potential of bringing novel bio-analytical applications, and they have a vast potential to realize low cost, efficient and reliable means for sensing and control [4-7]. The proposed biosensor will combine a fully leak-proof polymer microfluidic platform to an OLED light source. The device is capable of rapid in-situ detection of biological elements like concentrations of antibody using a minimum amount of fluorescence. In the rest of the paper, we describe the design of a multi-spectral, more integrated and cost-effective bio sensor that is capable of performing in-situ identification and measurement of the concentration of biological substances.
II. MICROFLUIDIC DEVICE AND FABRICATION

In a step-by-step approach, we started to work with a non integrated OLED used as a the light source and an independent microfluidic device. In this microfluidic device, the volume of samples needed to pass within the channel is very small. Hence, the amount of reagents and analytes used to test the sample is also quite small. In addition, the fabrication technique used to construct the microfluidic device is relatively inexpensive.

A. OLED Fabrication

OLEDs were fabricated on commercial ITO-coated glass substrates. ITO is lithographically patterned, chemically cleaned and treated by oxygen plasma then loaded into a vacuum chamber for organic material deposition. The organic stack structure consists of a copper phthalocyanine (CuPc) hole injection layer, a 4,4'-bis[N-(1-naphthyl)-N-phenyl-amino] biphenyl (α-NPD) hole transport layer and a tris-(8-hydroxyquinoline) aluminum (Alq3) electron transport and emissive layer. Finally a lithium fluoride and aluminum layer is deposited to serve as cathode. The organic layers and the cathode are deposited by thermal evaporation. The fabricated OLED chip has an effective area of 19 mm × 12 mm. Finally, OLEDs are encapsulated by a glass cover that prevents exposure to moisture.

B. Microfluidic device fabrication

A master template consisting of positive impression of the channels and chamber was fabricated. It consists of an inlet port and 2 rinsing ports, intersecting and leading to an outlet port. The intersecting region comprises a micro-chamber which facilitates enzymatic reaction. A re-usable polymer based microfluidic chip is prepared by pouring fully degassed PDMS elastomer and curing agent in the ratio 10:1 and cured at 75 ºC for 90 minutes. Cured PDMS consisting of open channels are then interconnected with the external world using polymer tubes and, later, irreversibly bonded to a thin flat PDMS sheet.

C. Integration and Detection

A filter was used to narrow down the excitation wavelength bandwidth so as to avoid interference of excitation intensity with the emitted signal range. It was found that the full width at half maximum of the OLED spectrum for a DC input voltage of 9 V was about 80 nm with a peak at 507 nm. Since the excitation and emission peaks of the fluorescence dye used in the experiments were 495 and 519 nm respectively, it was essential to select an excitation filter that would block noisy signals near the emission wavelength range. A high transmission sharp cutoff filter with band pass range between 460-500 nm was chosen as excitation filter and was placed between the OLED source and microfluidic chip. Fig.2 shows the OLED excitation with and without filter. Fluorescence tests were carried out for various concentrations of secondary antibody based on the immunoassay principle, with donkey anti-sheep IgG conjugates tagged to Alexafluor 488 (Molecular Probes) used as a sample. It has an excitation peak at 495 nm and emission peak at 519 nm. Phosphate buffer solution (PBS) was used as a buffer solution and a diluting agent. Three different concentrations of the sample were prepared: 1X (2000 µg/mL enzyme concentration), 2X (1000 µg/mL enzyme concentration) and 10X (200 µg/mL enzyme concentration), by further diluting the stock with with PBS. Isopropyl Alcohol (IPA) was used as a cleansing agent to rinse out the sample from the microfluidic channels for subsequent sets of experiments.

Fig. 3 illustrates how the light from the OLED is passed to the sample through the filter and how the transmitted signals are then collected and processed for the various concentrations of antigen.

D. Results

The device was tested to find the minimum level of concentration of fluorescing signal from the prepared samples. First PBS is passed through the pre-cleaned microfluidic chamber to initiate the experiments. The primary antibody which is already incubated to one of the two epitopes of antigen is then pumped through the inlet port and left to
immobilize inside the chamber. Unbound ligand is flushed out by rinsing the chamber with PBS again. 10X diluted tagged secondary antibody conjugate is then pumped into the microchamber and is left undisturbed to incubate with the primary antibody. Unbound antibody is flushed out by rinsing the chamber with PBS. The microfluidic device is then flushed with IPA and the methodology is repeated for secondary antibody with 2X, 1X concentrations and with only PBS.

The obtained results on fluorescence detection of tagged donkey anti-sheep IgG conjugates in the microfluidic chip using OLED excitation is given in Fig.4. It is seen that the fluorescence signal decreases with sample concentration and a minimum significant emission is observed at 10X diluted antibody containing 200 µg/mL donkey anti-sheep IgG conjugates. The minimum volume detected in the chamber containing 0.012 µl of sample had 2.4 ng of donkey anti-sheep IgG conjugates. The relative fluorescence units (RFU), or the ratio of excitation peak to the emission signal for various concentrations of antibody, shown in Fig. 5, confirm the decrease in emission signal with the increase in dilution.

These results lead us to investigate further the integration of the device, especially without using the optical filter, using an integrated organic photodetector and using intelligent signal processing techniques, such as a neural network, to process the raw data [3].

A. Emitter

There is the option of designing multiple emitters, each for a specific wavelength, using organic material. With this approach, the device can be tuned to specific applications by turning selected OLEDs ON sequentially. However, using this approach the sensor becomes larger in size. Our approach is then to use a single emitter with a broad spectrum – white light (WOLED). Hence, we intend to use one WOLED with an effective area of 3 mm × 3 mm which emits the whole spectrum from 350 to 750 nm. To keep the device size minimal, we put the WOLED at the center of a glass substrate as shown in Fig. 6. To fabricate the PDs and WOLED on the same substrate, we need to use different masks for different organic materials evaporation on the patterned ITO substrate. And finally we will use another mask for the cathode deposition.

B. Photo Detector

To make the integrated device, we need to grow several wavelength specific photo detectors (PD), made from different organic materials, on the same substrate. Each PD will detect light from the sample cell with a specific spectral response. Light falling outside is collected by the remaining PDs, which are not wavelength specific for the sample cell, will be noise. Initialization with the fluorophore dye, in the absence of the sample cell, will be done to calculate the noise level.
In order to test the detection principle we fabricated 3 different PDs from 3 different organic semiconductors - NPB (Naphthylphenylbiphenyl Diamine), PTCDA (3,4,9,10 perylenetetracarboxylic dianhydride), CuPc (Copper phthalocyanine) with ITO as the anode and Al as the cathode. Their photo currents were measured using a light source and a spectrometer (Science Tech 9055). The photocurrent responses for different wavelengths and bias voltages are shown in Fig. 7. Photo generated current peaks occurred at 415 nm, 490 nm and 615 nm respectively for each of the tested organic semiconductors. With a small bias voltage applied to the PDs the generated photo current is significantly increased. The exact value of bias needed for any specific PD depends on the sample cell. We are presently investigating other materials in order to build other organic PDs which will generate high photo currents with high and sharp peak at other wavelengths. Ultimately, the spectral signature from all the PDs will be routed to a current-to-voltage converter and amplifier. The data acquisition and processing could then take place by using a neural network for concentration measurement as described in [3].

IV. CONCLUSION

We described a general purpose, cost-effective multi-spectral fluorescence measurement system to identify and measure the concentration of samples with a neural network for data processing. At first, a single spectral measurement system – the polymer based microfluidic platform was designed, developed and successfully integrated with excitation optical filter and detector for bio-sensing applications. The device was tested to detect anti-sheep IgG conjugates tagged to Alexafluor 488 using a fluorescence based immunoassay technique. The minimum detectable concentration of the antibody was identified experimentally at an enzyme concentration of 200 µg/mL. Minimum amount of fluorescence detected was 0.012 µl with 3 ng of IgG conjugates. Thus, to extend the applicability of OLED based sensor, we designed general purpose, multi band, more integrated device. We found that wavelength specific organic photo detectors can be fabricated from single emissive layer of monomer material. These PDs produced sharp and high photo generated current peaks.

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