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Integrated System Based on Thin Film Technologies for Cell-Based Bioluminescence Assays †

Domenico Caputo [1,*], Giulia Petrucci [1], Valeria Di Fiore [1], Alessio Buzzin [1], Marco Nardecchia [1,2], Luca Cevenini [3], Elisa Michelini [3], Mara Mirasoli [3], Aldo Roda [3], Nicola Lovecchio [1], Francesca Costantini [1,2], Augusto Nascetti [2] and Giampiero de Cesare [1]

[1] Department of Information Engineering, Electronics and Telecommunications, Sapienza University of Rome, Rome, Italy; petrucci@diet.uniroma1.it (G.P.); valeriadf89@gmail.com (V.D.F.); b.alessio1987@gmail.com (A.B.); marco.nardecchia@uniroma1.it (M.N.); nicola.lovecchio@uniroma1.it (N.L.); francesca.costantini@uniroma1.it (F.C.); giampiero.decesare@uniroma1.it (G.d.C.)

[2] School of Aerospace Engineering, Sapienza University of Rome, Rome, Italy; augusto.nascetti@uniroma1.it

[3] Department of Chemistry "G. Ciamician", Alma Mater Studiorum—University of Bologna, Bologna, Italy; luca.cevenini5@unibo.it (L.C.); elisa.michelini8@unibo.it (E.M.); mara.mirasoli@unibo.it (M.M.); aldo.roda@unibo.it (A.R.)

* Correspondence: domenico.caputo@uniroma1.it; Tel.: +39-(0)6-44-585-832

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Abstract: This work presents a miniaturized lab-on-chip system suitable for monitoring the activity of living cells through the on-chip detection of their bioluminescence emission. The system integrates amorphous silicon diodes, acting as temperature and light sensors, and indium tin oxide film, acting as heater, on a single glass substrate. During its operation, the glass is thermally and optically coupled to the investigated cells and electrically connected to an electronic board, which controls the lab-on-chip temperature and monitors the sensor photocurrents. The proposed lab-on-chip is particularly attractive for the development of portable cell-based biosensors useful for biological activity monitoring as well as for cell cytotoxicity evaluation.

Keywords: lab-on-chip; thin film technology; amorphous silicon; bioluminescence; cell bioreporter

1. Introduction

Labor-intensive and complex laboratory procedures can be simplified through lab-on-chip (LoC) systems, which integrates several laboratory functions on a single chip. The advantages of LoC include reduced sample and reagent consumption, fast detection times, miniaturized size, possibility to perform the analysis on field as well as no need for expert operators and specialized personnel [1]. These benefits are attained by integrating microfluidics with electronic devices in a compact system.

A critical point in achieving a successful miniaturization is the use of proper techniques for the biomolecules detection and for providing the thermal energy needed for the analytical treatments. Among the different detection methods [2,3], those based on optical techniques are the most in vogue due to their high sensitivity, robustness and reliability [4]. Several solutions have been proposed including the use of photosensors based on organic or inorganic materials [5,6]. On the other hand, in order to avoid bulky, heavy heating blocks [7], different microheaters have been developed [8,9] to ensure a uniform temperature profile.

Within this framework, we integrate on a single glass substrate different thin film technologies in order to develop a LoC system suitable for on-chip thermal treatments of living cells and simultaneous on-chip detection of their bioluminescence (BL) emission. Bioluminescence reactions are characterized by high quantum yield emission and low background, which results in high

detectability and sensitivity of BL-based assays. In addition, as BL does not require an external excitation light source, it represents an ideal detection principle for miniaturized sensors, lowering weight, price, size and complexity of integrated systems [10,11].

2. Materials and Methods

2.1. System Structure

The developed system integrates hydrogenated amorphous silicon (a-Si:H) diodes and indium tin oxide (ITO) thin film heater on the same glass substrate. The a-Si:H diodes, deposited on the bottom glass side, work as both temperature sensors and photosensors, while the ITO layer, deposited on the top glass side, acts as transparent heating source. Figure 1 depicts the integrated thin film devices and their optical and thermal coupling to the sample (e.g., whole-cell bioreporters).

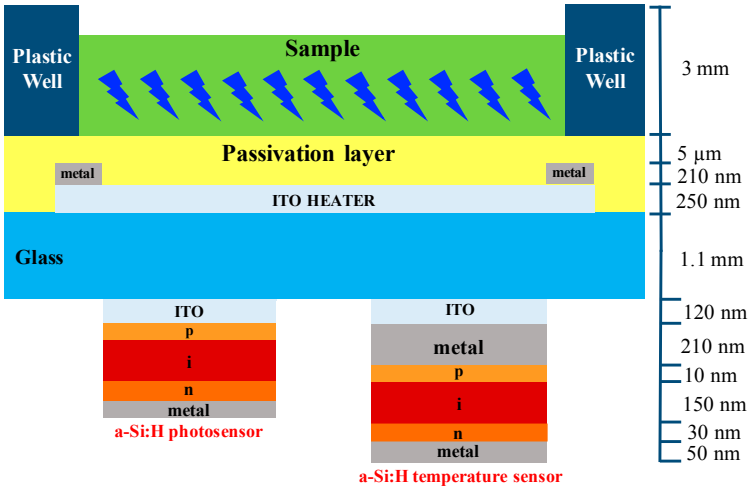


Figure 1. Cross section of the proposed system: The glass substrate hosts the transparent ITO heater on the top side and the a-Si:H sensors on the bottom side. The photosensor (**left**) and temperature sensor (**right**) differ for the bottom contact: ITO for the photosensor and a stack of ITO and metal (TiW) for the temperature sensor. The plastic well, with transparent bottom, contains the biological sample where the BL emission (blue arrows) occurs.

The system allows: (i) thermal treatment of the cell by powering the thin ITO film. Applying a voltage to the thin film heater, an increase of the sample temperature is induced by conduction heat transfer; (ii) monitoring of the sample temperature thanks to the a-Si:H temperature sensors [12]; (iii) detection of the BL signal emitted by the cells through the a-Si:H photosensors. The control of the sample temperature is performed through a dedicated electronic board connected to the heater and to the a-Si:H temperature sensors [13]. The transparency of the thin film heater is crucial for the correct transmission of the emitted light to the a-Si:H photosensors. The metal layer covering the ITO contact acts as light shield for the temperature sensor.

2.2. Biological Sample

Saccharomyces cerevisiae yeast strain (BMA64-1A) was genetically engineered by introducing a reporter vector for the constitutive expression of the NanoLuc luciferase, emitting at 460 nm [14]. Yeast bioreporters were routinely grown in Synthetic Complete (SC) liquid medium without leucin in an orbital shaking incubator at 30 °C, 250 rpm. Cells were grown in SC medium and serially diluted. Yeast bioreporters (20 µL) were poured into a plastic well with transparent bottom (Figure 1), obtained using a desktop 3D printer, and placed over the a-Si:H sensor. Bioluminescence emission kinetics (1000 readings with 2 s integration time) were acquired after addition of the specific BL cocktail (10 µL) containing 10 µM furimazine. The limit of detection (LOD) was calculated as the

number of yeast cells which provides a signal corresponding to that obtained with the blank (SC medium and BL cocktail only) plus three times its standard deviation.

2.3. Fabrication of a-Si:H Sensors and Thin Film Heater

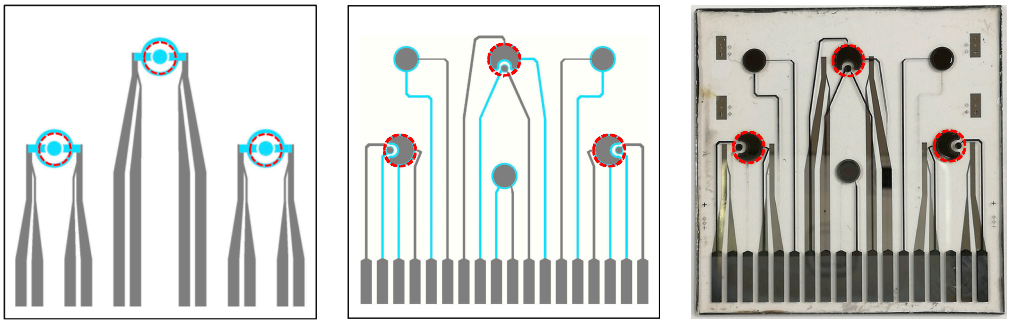
The a-Si:H diodes are p-i-n structures deposited by Plasma Enhanced Chemical Vapor Deposition in a three-chamber high vacuum system (GSI, Denver, CO, USA). An accurate design of the thickness and energy gap of the layers have been carried out to match the photosensor responsivity with the BL emission spectrum. In particular, we fabricated a p-type doped amorphous silicon carbide (a-SiC:H)/ intrinsic a-Si:H/ n-type doped a-Si:H with thickness equal to 10, 150 and 30 nm, respectively. Details of the deposition parameters can be found in [15].

The ITO film is deposited by magnetron sputtering (MRC, Orangebourg, New York, NY, USA).

3. Results and Discussion

3.1. Heater and a-Si:H Sensors

Heater geometry and materials have been optimized, by using COMSOL Multiphysics, to satisfy requirements of temperature uniformity over the active area of the thermal treatment and negligible cross talk between the different heaters. The optimized spatial arrangement is reported in Figure 2a, where three independent heaters have been distributed on a 5 × 5 cm^2 glass substrate. Each heater has a circular shape with an active area of 0.18 cm^2.



(**a**) (**b**) (**c**)

Figure 2. (**a**) Top view of the spatial positioning of the thin film heaters; (**b**) Top view of the spatial positioning of the photosensors (moon-like shape) and temperature sensors (small circular shape) aligned with the heaters; (**c**) Picture of the fabricated device (a-Si:H sensor side). The red dashed circles indicate the active area of the BL emission.

The circular heathers (Figure 2a) have been aligned with the light and temperature sensors on the other side of the glass substrate (Figure 2b). The moon-like shaped are the photosensors, while the smaller, circular shape are the temperature sensor. This configuration maximizes the photosensor active area for collecting the BL signal. Three additional photosensors (large circular shapes in Figure 2b) have been located on this side of the glass for general purpose.

Afterwards, heaters and thin film sensors have been fabricated using standard microelectronic technologies [15]. Figure 2c shows a picture of the fabricated device. A careful tuning of sequence and parameters has been carried out to achieve the compatibility of the different technological steps.

In order to confirm the simulation results and the effectiveness of the utilized heater geometry, we measured the temperature distribution on the upper side of the glass (Figure 1) by using a FLIR A325 thermocamera. We found that the measured average temperature on the active area, aligned with the heater, was 37 ± 0.3 °C. The corresponding modeled temperature distribution was 37.1 ± 0.3 °C showing therefore an excellent agreement with the experimental data.

Regarding the a-Si:H sensors we found that the photosensors present a responsivity equal to 210 mA/W at 460 nm (the wavelength at which the peak of the BL emission occurs), while the

temperature sensors have a sensitivity around 3.2 mV/°C. These values are comparable with those achievable with the state of the art crystalline silicon sensors.

3.2. Acquisition of BL Emission from Living Yeast Bioreporters

The suitability of the developed a-Si:H photosensors for the detection of light emission from BL whole-cell bioreporters was evaluated using the genetically engineered yeast cells described in Section 2.3. The cells were serially diluted from 107 cells down to 100 cells/well, and BL emissions were acquired after addition of the BL cocktail containing furimazine. The use of the bright NanoLuc luciferase as reporter combined with the highly sensitive a-Si:H photosensors provided a limit of detection of 1500 ± 200 cells with a linear response of three order of magnitude up to 10^6 cells.

4. Conclusions

We have presented an integrated LoC system, based on thin film microelectronic technologies, designed to monitor and control the activity of living cells. In particular, a-Si:H sensors and thin film heaters have been fabricated on the same glass substrate in order to achieve a compact system able to control the cell temperature and detect the BL emission with high sensitivity. The developed configuration allowed the on-chip detection of genetically engineered BL yeast bioreporters down to 1500 ± 200 cells with a linear response up to 10^6 cells.

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Conflicts of Interest: The authors declare no conflict of interest.

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