Miniaturized Wireless Cell Spectrophotometer Platform in Visible and Near-IR Range

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Abstract

In this paper, a new miniaturized wireless cell spectrophotometer is presented. This system can scan a sample, detect incoming light power and transmit corresponding data to a base station for further analysis in the range of 340 nm to 850 nm. *In vitro* measurement results with VERO E6 cells tagged with DAPI and Alexa Fluor488 are presented to demonstrate its performance. The proposed system uses two small Lithium-ion batteries that provide a 7.4 V supply voltage. The system's low power consumption (88 mW), its minimal use of hardware resources, and its total weight of 17 g incorporated into a small wireless platform make the proposed device suitable for real-time implementation in most common low-power cell spectrophotometer applications.

I. INTRODUCTION

Increasing demand for monitoring and detecting the chemical composition of different materials using optical interfaces emphasizes the importance of the qualitative and quantitative data collected from each of them. Having such information for different materials can help in a vast area of applications [1, 2, 3], including identifying toxic substances, biopsy, testing foods and water quality, tracking environmental changes, and monitoring health, each of which has many subsets. For example, health monitoring includes the course of treatment, chronic disease management, pathogen detection, and early disease detection. One of several existing identification methods, optical spectroscopy has gained much attention because of its proven capability to provide a wealth of significant data from scattered, reflected, emitted, and absorbed light [1, 4]. The light emitted from each material serves almost as a fingerprint; each exhibiting a particular pattern. Therefore, spectroscopy has the potential to allow qualitative and quantitative detection of different materials within various noninvasive or minimally invasive methods.

Fig. 1 depicts the typical structure of a conventional bench top spectroscopic device, of the type used for food inspection, biometry, and various light level measurements [1, 2]. As it can be seen in Fig.1, the incoming light from thesample passes through optical components including lenses and a grating to reach the photodetector array. The lens collimates the incoming incident light onto the grating component, which diffracts it onto an array of different photodetectors. The photogenerated current which is converted into a voltage signal through an analog frontend circuitry, digitized, and then sent to a computer. However, such a system is too bulky for implantable applications.

Much effort has been devoted to creating miniaturized spectroscopy devices over the past number of years due to the increasing demand for battery-operated portable equipment and power-saving circuits [3, 4]. Following these efforts, in this paper, a low-cost, low-power, miniaturized optical platform is developed using discrete, commercial, offthe-shelf components. The proposed cell spectrometer is intended for sensing optical signals emitted from cells tagged with Alexa Fluor488 and DAPI. VERO E6 (African green monkey) fixed cells were used to do these *in vitro* experiments. The implemented device works within the UV, visible and Near-Infrared (NIR) range, namely the wavelengths from 340 nm to 850 nm. The paper is organized as follows. Section II presents an overview of the proposed design and section III describes the system design. Section IV is provided to show the performance of the proposed device. Section V explains the materials and methods that are used for *in vitro* experiments. Section VI discusses the in vitro results. Finally, the last section provides a conclusion to this paper.

II. SYSTEM OVERVIEW

Fig. 2 (a) illustrates a block diagram of the proposed miniature wireless optical spectroscopy system. This device uses compact multilayer printed circuit boards (PCBs) that are stacked and joined together with a rigid custom-designed connector. Using a multistage PCB in this design helps to decrease the size of the final device. The spectroscopy system detects the emitted light from a sample under test (SUT) using a fiber with a 1-mm diameter. The fiber is used to specify the area being tested. It also helps to prevent the leakage of light into the sensor slit, which in turn can improve the quality of the detected signal.

Furthermore, using such a fiber supports the implantation of the prototype for long-term detection in freely-moving live testing animals. A sensor cap prevents the leakage of light from the input slit of the sensor and is also responsible for holding the fiber. The detected light is processed by analog and digital building blocks, which generate digital code representing the input signal information. The recorded data is transmitted to a base station which is located a few meters away from the experiment. As shown in Fig. 2 (b), the dimensions of the PCB layers are 3 cm × 2.4 cm × 1 cm with a total weight of 17 g. The entire wireless spectroscopy sensor interface is powered by two 3.7 V, 100 mAh, 2.1 g Lithium-ion batteries (Model 051417, MYD Technology) which provide 7.4 V whenconfigured together. After sending the data to the computer, the interface provided by MATLAB shows us the intensity of each wavelength detected by the device. This interface designed with MATLAB visualizes the detected light intensity versus wavelength. Light intensity is normalized from 0 to 1 for the wavelengths from 340 nm to 850 nm.

III. SYSTEM DESIGN

As shown in the block diagram in Fig. 2 (a), the proposed prototype consists of multiple submodules, which are constructed using discrete commercial off-theshelf components. These are selected to minimize power consumption and space occupancy to create a miniaturized, low-power device. The device is divided into two main parts consisting of optical, and electrical circuits that are summarized in the following subsections. The optical part provides the grating and photodetector [2] and the electrical part converts the incoming signal into a digital signal which is then transferable through digital communications using a wireless transmitter.

A. Mini-spectrometer

Optical parts of the spectroscopy device, as shown in Fig. 1, serve to diffract the incoming light and detect the received power of each wavelength. For this purpose, a highly sensitive Hamamatsu mini-spectrometer (C12880MA) is used; its structure is shown conceptually in Fig. 3. After passing through the sample, the light enters the device by the input slit. Then, the reflective concave blazed grating diffracts the incoming light to its constitutive wavelength. The diffracted light reaches the photodetector array, which is placed inside the mini-spectrometer, as shown in Fig. 3. The dimension of the fingertip spectrometer is 20.1 mm × 12.5 mm × 10.1 mm, and it has a weight of 5 g. The resolution of this device is 15 nm, which is sufficient for the detection of cells tagged with Alexa Fluor488 and DAPI [2], and it supports wavelengths from 340 nm to 850 nm.

To design an appropriate interface between the mini-spectrometer and the electronic component, the timing of the output data of the mini-spectrometer must be considered. The Hamamatsu photodetector sends the detected light within multiple sets of data at the output; each one of the sets takes a 288-clock period. A signal, which is called EOS (End Of Signal), turns to one from zero at each time to indicate that one set of data is finished. Then, an 87-clock period is needed to start the next set of data. Therefore, each set needs a 375-clock period. The device works at a 0.2 MHz to 5 MHz clock frequency. Finally, the output signal of the mini-spectrometer is buffered and lowered by electronic circuits to be prepared for the next set.

B. Control, transceiver, and power unit

The next part consists of a power management unit (PMU), a microcontroller (MSP430), and a wireless module (NRF24L01P). Power is supplied by two 3.7 V, 100 mAh and 1.1 g Lithium-ion batteries, which can provide 7.4 V of power. This voltage, using two regulators, provides fixed voltages at 5 V (VDD) and 3.3 V (VCC) for the whole circuit. An nRF radio transceiver from Nordic Semiconductors is used to transmit data from the proposed device to the base station. This module operates at a 2.4-GHz center frequency. Finally, a microcontroller (MSP430F5529IPNR) from Texas Instruments provides control signals of the whole sensor. The microcontroller receives the data set from the minispectrometer, and then, using the SPI bus and ADC units, converts the received data into an appropriate signal for the nRF module. All of these circuit parts work with a 3.3 V voltage supply and consume 33 mW of power.

IV. TEST RESULTS

The system's performance was evaluated by using different coloured LEDs whose wavelengths ranged from 467 nm to 620 nm. The LEDs were connected to the fiber tip; the light was then conducted through the fiber to the spectroscopy device. MATLAB GUI shows the emission intensity of each LED versus its wavelength as shown in Fig. 4. In Fig. 4, the peak wavelength of each LED is also given. The results show that this prototype is able to detect the expected wavelength of each colour.

To demonstrate the precision of the proposed device, orange and red LED can be used as examples. As shown in Fig. 4, the wavelength of the orange LED has a peak (λ_{peak}) at 601 nm and the red LED has a λ_{peak} at 614 nm. These results show 13-nm precision, which is 2 nm better than what we expected from the Hamamatsu mini-spectrometer datasheet. This result shows that the proposed setup meets the expected precision. To show the performance and precision of the device, the white LED was used to do another LED test with the same procedure. The result shows the exact plot that is available in the datasheet for white LEDs. The output of the white LED is also shown in Fig. 4. Such results prove that this prototype can be utilized to detect the absence or presence of a cell, which is discussed in the following sections. Furthermore, decreasing and increasing the 3V supply voltage of LEDs results in lower and higher levels of amplitude for detected emission intensity, which in turn supports the prediction that this device can also detect the concentration of SUT.Table I summarized the performance of the proposed prototype.

V. MATERIALS AND METHODS

The device's ability to detect wavelengths from 340 nm to 850 nm was assessed using two different fluorescent tags, namely 4′,6-diamidino-2-phenylindole-DAPI (Cat. No. D1306, Thermofisher scientific, Canada) and Alexa Fluor 488 (A-11059). The fluorescent dyes were diluted in 1 mL of Phosphate Buffered Saline (PBS) in a 12-well plate (Cat. No. 83.3921, Sarstedt, Canada). The plate was then placed on the microscope (Olympus IX81) and the wells containing Alexa Fluor488 and DAPI were illuminated by light sources at 495 nm and 358 nm, respectively, using Olympus IX81 lamps. The fiber of the spectroscopic device was secured to the top of the well as it was illuminated by the light source to detect the light emitted by the tags. The wavelength of the emitted light was recorded on a graph. To further expand on the application of this device, its ability to detect fluorescent signal from fluorescently tagged cells was studied. For this purpose, VERO E6 cells (African green monkey) were fixed on a coverslip. The fixed cells were then hybridized with anti-tubulin antibody as the primary antibody followed by Alexa Fluor488 as the secondary antibody. The cells were then incubated with DAPI for nucleus staining. The coverslips were placed on a glass slide and observed under the microscope. Moreover, to detect the light emitted from tagged cells, the slides were illuminated by the microscope's light source while the fiber of the spectroscopic device was touching the upper surface of the slide for detection. The proposed device recorded the wavelength of the emitted light. This data was then analyzed and visualized with MATLAB. The shape of the cells marked with the two aforementioned tags was captured by the microscope and is shown in Fig. 5.

VI. *IN VITRO* MEASUREMENTS

The protocols mentioned in section IV were performed to prove the performance of the proposed device in an *in vitro* environment. As a primary test, the wavelengths emitted by two different fluorescent tags were assessed. For this purpose, the fluorescent tags were diluted in 1X PBS solution in different wells of a 12-well plate. The wells were then illuminated at the respective excitation wavelength for each fluorescent tag: 495 nm for Alexa Fluor488 and 358 nm for DAPI. 1X PBS was used as a control for the experiment.

As shown in Fig. 6 (c), when PBS was illuminated at 495 nm, the proposed device detects the incoming light with a peak at 495 nm that corresponds to the input light source. Whereas, when the same wavelength was used to illuminate the well containing the fluorescent tag Alexa Fluor488, two peaks were detected by the device, one at 495 nm from the light used for excitation and one at 519 nm from the emitted light (Fig. 6 (e)). The same results were observed for DAPI, with an observed peak at 358 nm for the control and two peaks for the DAPI-containing well that correspond to 358 nm, the wavelength of the light source, and 461 nm, the light emitted by the excited DAPI molecules (Fig. 6 (d) and Fig. 6 (f)). These results confirm that our proposed device is capable of detecting different wavelengths of light emitted by fluorescent tags. Therefore, to consider the application of our device in greater detail, we assessed its ability to detect the light emitted by fluorescently tagged cells. Accordingly, VERO E6 cells were fixed on a coverslip and then hybridized using an anti-tubulin antibody followed by the secondary antibody Alexa Fluor488 with the maximum absorption at 495 nm. The cells' nuclei were also stained with DAPI with the maximum absorption at 358 nm. For the tagged cells, as shown in Fig. 6 (g) for Alexa Fluor488 and Fig. 6 (h) for DAPI, the proposed device could successfully detect the light emitted by cells tagged with Alexa Fluor488 when illuminated at 495 nm, and DAPI when illuminated with 358 nm. The designed MATLAB interface plots a figure based on the data received data from the prototype. These plots, as shown in Fig. 6 (g) and Fig. 6 (h), present two peaks to show the source light and emitted light from tagged cells. The peaks correspond to 495 nm (Fig. 6 (g)) and 358nm (Fig. 6 (h)) as the wavelength of the light source, and 519 nm as the emitted light from Alexa Fluor488 (Fig. 6 (g)) and 461 nm as the emitted light from DAPI (Fig. 6 (h)). The similar results were observed in the Alexa Fluor488 and the DAPI nucleus stains indicating the effectiveness of our device in detecting fluorescent light emitted by cells.

VII. CONCLUSION

A wireless optoelectronic interface for detecting VERO E6 cells tagged with DAPI and Alexa Fluor488 using commercial off-the-shelf components and a mini-spectrometer (C12880MA) has been presented. The performance summary of the proposed device has been shown in Table I. Altogether, based on our observations, the proposed setup using a miniaturized device with a proper interface in MATLAB can be considered a low-power option for a cell imaging system. Notable features include the device's ability to detect light emitted by cells, as well as its low power consumption, light weight, and easy handling. Its size and light weight make the device very easy to incorporate into any settings. It is worth mentioning that the setup functions wirelessly with a battery and can send the data to the base station independent of any connections. Moreover, the designed cap on the Hamamatsu mini-spectrometer that cuts off light leakage into the sensor and the use of a fiber for the detection of a particular $500\times500\mu m^2$ area are other noteworthy characteristics of this device. Its miniaturization would decrease power consumption, costs, and occupied space, as well as offer more flexibility and expandability for use in several different applications.

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Table 1-Performance Summary

Parameter	Value
Power	88mW
Supply Voltage	7.4V
PCB size	3cm × 2.4cm × 1cm
Total weight	17g
Wavelength Range	340nm to 850nm
Resolution	13nm
Sensing area	500×500 μm^2

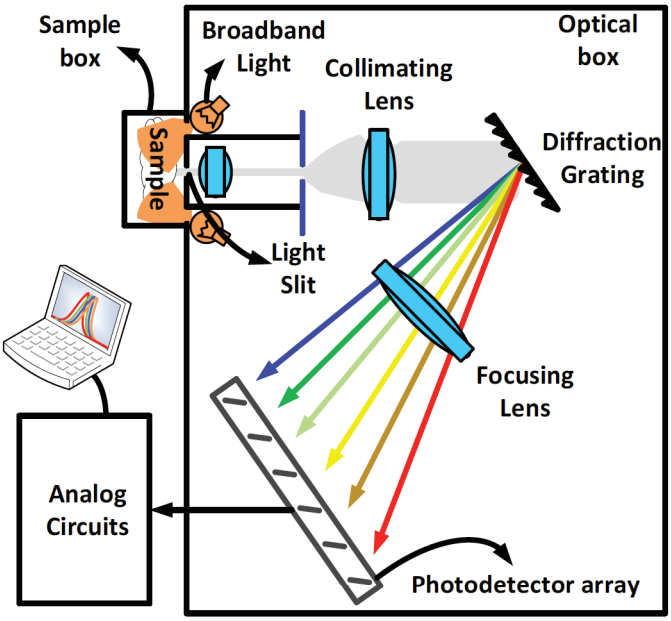

Fig. 1. Conceptual structure of a conventional spectrophotometric device.

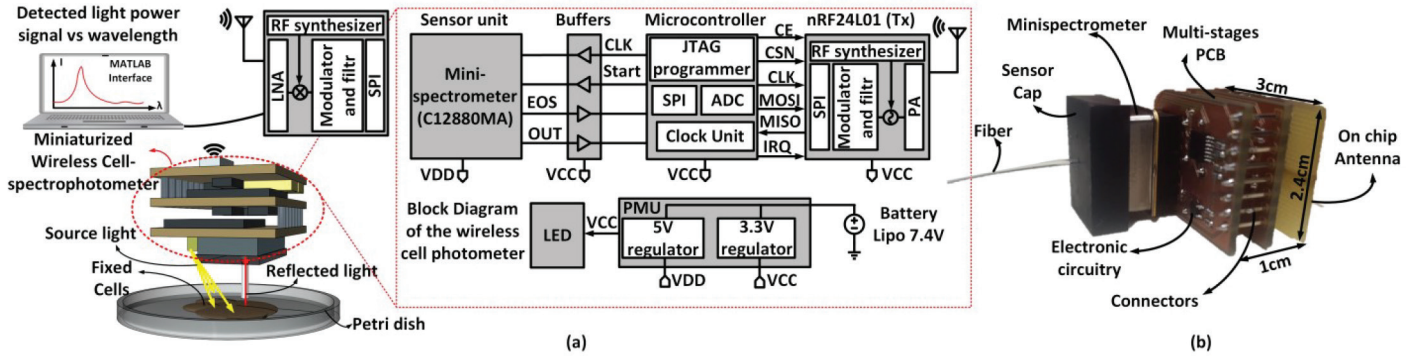

Fig. 2. a) Conceptual image and block diagram of the proposed system. The system can detect the emitted wavelengths from tagged cells between 340 nm to 850 nm. b) The mini-spectrometer, optical fiber, and different components enclosed within a lightweight wireless prototype that can be used as a cell spectrophotometer are shown in a photo of the prototype.

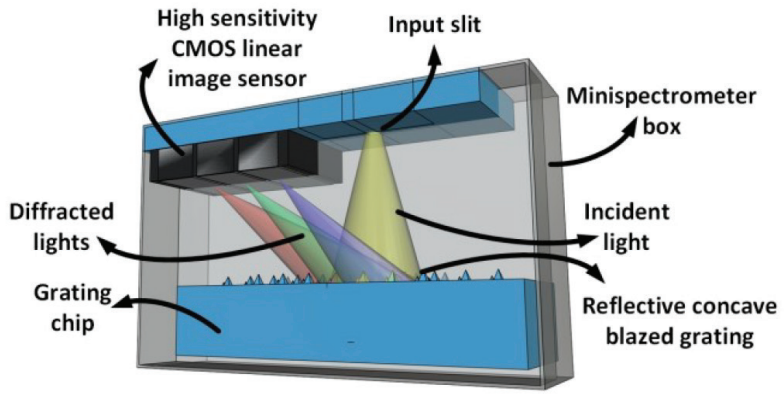

Fig. 3. Details of mini-spectrometer structure inside the sensor box.

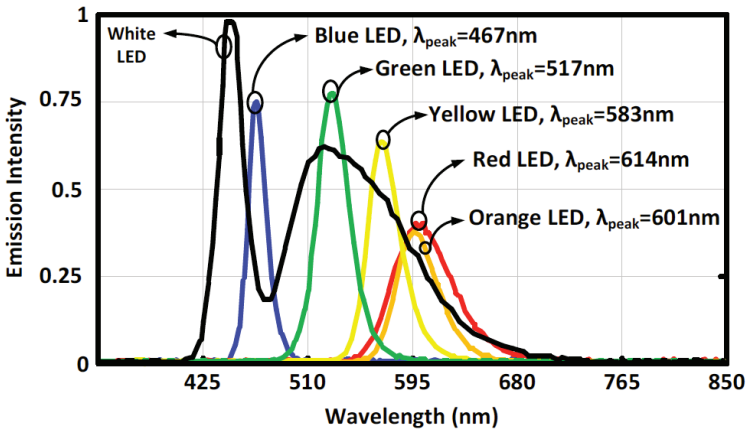

Fig. 4. Detected emission intensity versus wavelength of several LEDs by proposed device. The plot shows the shape of each colour and its corresponding detected value for peak wavelength (λ_{peak}). It also shows the white LED's shape which is exactly like the white LED's datasheet.

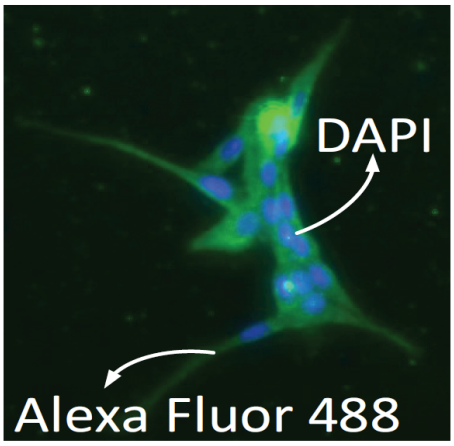

Fig. 5. Test cells tagged with Alexa Fluor488 and DAPI, shown in green and blue, respectively. This image is taken in the Lab. by an Olympus IX81 microscope from experimented tagged cells. It shows that the core and body of the cells are marked by different tags.

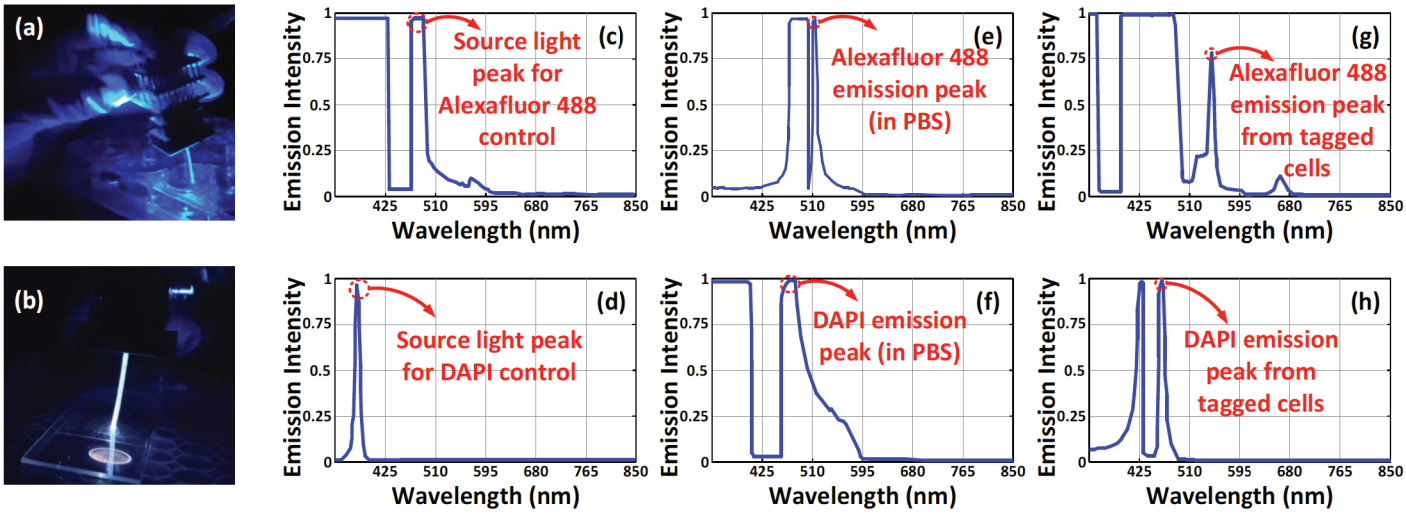

Fig. 6. The results and setup for in vitro experiments. The upper images are for Alexa Fluor488 and the bottom images are for DAPI. (a) image of setup used to detect the Alexa Fluor488 diluted in PBS. (b) image of setup used for detecting the tagged cells with DAPI. The other diagrams indicate the intensitty of the emitted light from samples that is detected by the proposed device versus wavelength for (c) Control Alexa Fluor488, (d) Contol DAPI, (e) Alexa Fluor488 diluted in PBS, (f) DAPI diluted in PBS, (g) VERO E6 cells tagged with Alexa Fluor488, and (h) VERO E6 cells tagged with DAPI.