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VERTICALLY INTEGRATED OPTICAL TRANSDUCER FOR BIO-PARTICLE DETECTION

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Abstract

An optical transducer with vertical structure integrated with MEMS microfluidic device is developed for the detection of bio-particle. The system consists of optical detection part; electronics control part and microfluidic part. Integrating of these parts, using optical transducer as a label-free method can provide the diagnosis of low volume biological samples as well as leading to direct detection with no significant change in physical forms of the samples. A collimated light source of deep UV-AlGaN based LED and a SiC photodiode operating at 260 nm are utilized as a light transmitter and detector, respectively. The principle of bio-particle detection is based on absorption of ultraviolet (UV) range of DNA biological samples extracted from a Caco-2 cell in a fluidic medium. The outcomes show that the signal is detected by the SiC photodiode for the rise time of $\tau_r \sim 118.1371$ µs which shows sufficient response time to enable the detection of the bio-particle. Testing on various samples showed that the detector has no response to non-absorbance samples such as deionized (DI) water, Tris-EDTA (TE) buffer and protein samples. However, a significant outcome in the detection of a Caco-2 cell line with the absorbance coefficient of average 0.08 a.u was achieved.

Keywords: Optical transducer; MEMS device; PDMS microfluidic; UV LED; SiC photodiode

1. Introduction

One of the principles to detect the biological particles accurately in MEMS devices is employing an optical transducer. This method was recently identified to have a higher potential for real-time detection of biological and chemical particle

Nomenclatures

I_{ph}	Photocurrent, A

Greek Symbols

τ_r	Rise time, µs

Abbreviations

DI	Deinonized
DNA	Deoxyribonucleic Acid
LED	Light Emitting Diode
MEMS	Microelectromechanical Systems
PDMSS	Polydimethylsiloxane
SiC	Silicon Carbide
TE	Tris-EDTA

samples because of its extremely sensitive and label-free nature, as well as its fast response [1] . Some bio-particles, such as nucleic acids have a strong absorption in the UV region range between 240 to 275 nm, which make them an excellent candidate as a medium in UV base bio-particles sensors or detectors. For instance, deoxyribonucleic acid (DNA) has a strong absorption at 260 nm whose property can be practiced to determine its concentration [2, 3].

Several reports on the direct measurement of UV absorbance using optical transducer were reported [4-6] in which the microsystem consists of a UV-LED as the light source can transmit light at the precise wavelength through a quartz container of DNA. In addition, this kind of UV optical transducer for absorbance measurement have been developed with various configurations and applications such as detection of aromatic hydrocarbons in water [7], determination of nitrite and total nitrite in water [8] and photometric device in capillary electrophoresis [9].

The integration of optical transducer into a micro system has been realized previously. The advantage of the integrated detection system is that it can provide portable sensing or fast diagnosis with the only small volume of bio-particle. Most of the reported devices were built in macro scale, which are unable to fulfill the demand for portable and micro size detecting device. The bio-particle samples such as bovine serum albumin [10], chemical and biological species [11, 12] were successfully detected and measured using absorbance method, which also can be implemented to study single molecules and enzymatic reactions [13]. It was likewise reported that optical detection provides faster, more reliable approach, which can improve the accuracy and safety of the particles.

In recent years, MEMS microfluidic devices based on polydimethylsiloxane (PDMS) polymer have been developed and integrated with the optical transducer to perform bio-particles detection devices [14-20]. PDMS is one of the most widely used material to fabricate microfluidic devices, due to its biocompatibility and transparency from 240 to 1100 nm.

In this work, a highly selective and sensitive optical transducer based on light emitting diode (LED) and a photodiode operates in a UV light region is developed for direct measurement of biological samples in a transparent

microfluidic channel. A deep UV LED with the wavelength spectrum that match the spectrum absorption of the sample is used as the light source. A microliter sample in the chamber detection of the microfluidic channel absorbs the light once it is striking in without any waveguide. The rapid response photodiode having a similar peak wavelength with the sample's spectrum absorption sensed the induced photocurrent due to the absorption process. The voltage change is then dealt with by the signal conditioning and can be observed on an oscilloscope. Using this label-free and selective vertical optical transducer integrated with PDMS microfluidic device, a biological and chemical bio-particle can be detected and diagnosed.

2. Methods

2.1. System construction

The schematic diagram of the integrated optical detection system with the vertical structure optical transducer is shown in Fig. 1. The transducer consists of a deep UV-LED-AlGaN (UVTOP255-BL-TO39) light emitting diode located perpendicular to a Silicon Carbide (SiC) (SIC01L-5-TO5) UV photodiode incorporating the signal conditioning circuit and a transparent microfluidic device.

Input signal for the UV-LED driven by the LED driver is a quasi-continuous wave signal (quasi-CW) generated by a digital timing and control circuit. This digital timing and control circuit monitor the operation of the UV-LED by controlling the frequency, sequences and the duration of the light emission. The light signal is received by a low dark current, high speed and low noise Silicon Carbide (SiC) SIC01L-5 UV photodiode with an active chip area dimensions of 1 x 1 mm^2, 0.96 mm^2. The output signal is displayed on an oscilloscope (LeCroy WaveSurfer 424 200 MHz).

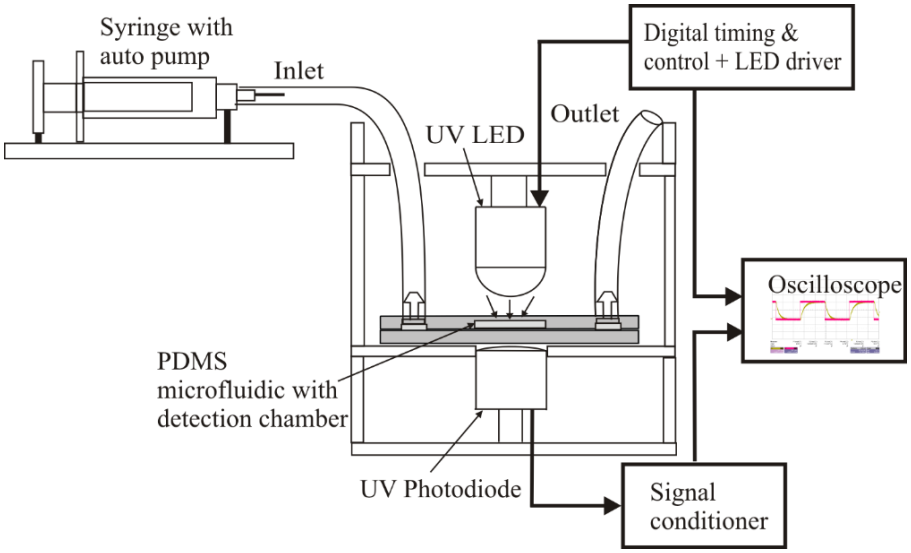


Fig. 1. Schematic diagram of the experimental setup for measuring light absorption of bio-particle samples.

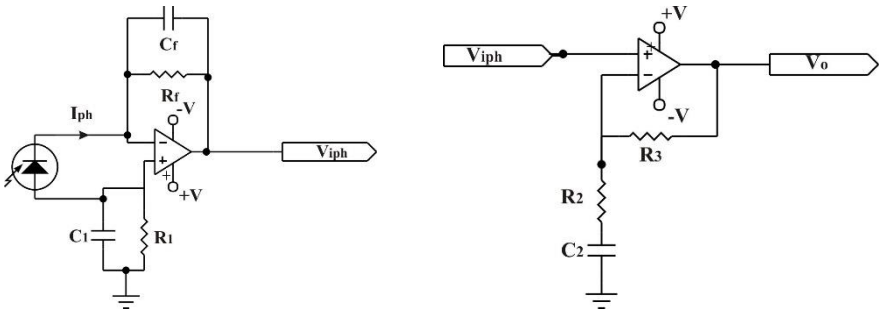
The light signal emitted from the LED with a modulation frequency of 1 kHz is traveling along a microfluidic chamber and directly interacts with the bio-particles. The microfluidic device consists of a channel, fluidic chamber, inlet and outlet. It is made of polydimethylsiloxane (PDMS) material and fabricated by using standard MEMS processes. The detailed process for the fabrication of the microfluidic channel was reported in our previous report [21]. PDMS is chosen as the material for the microfluidic due to its optically transparent to the UV light [22].

The remaining light signal detected at photodiode is attenuated and converted into a photocurrent that is influenced by the amount of sample particles in the chamber. Since, the photocurrent detected by the photodiode is very low, the converted signal has to be amplified, conditioned and converted to a proportional voltage by the signal conditioner and amplifier.

2.2. Working principle

The device employed a deep UV-LED that was chosen in accordance with bio-particle sample spectrum absorption in UV region. The LED was driven by an input signal control circuit with a quasi-CW modulation frequency of 1 kHz and driving DC at 20 mA. The LED is pulsed ON for 500 µs and OFF for another 500 µs with a duty cycle of 50 %. With this configuration, the measurement of light intensity by the photodetector can be carried out both in a dark or light condition.

The UV light absorbed by the bio-particle sample was assessed by a SiC photodiode that was configured in the photovoltaic mode in the pre-amplifier stage of the signal conditioner circuit shown in Fig. 2(a). This configuration converts a variable input photocurrent in a form of modulated light signals (On and OFF transient response) to a proportional voltage, where this photovoltaic mode can minimize the dark current.



(a) Pre-amplifier with low pass filter.

(b) Amplification with high pass filter.

Fig. 2. Signal Conditioner circuits of SiC photodiode in photovoltaic mode.

The relationship between the two variable; voltage photocurrent (V_{Iph}) and photocurrent (I_{ph}) is given by Eq. (1):

$$V_{Iph} = I_{ph}(R_f + R_1) \tag{1}$$

where V_{Iph} is the voltage corresponding to the photocurret, I_{ph} is photocurrent, R_f is a feedback resistor and R_1 is a resistor tied to the anode of the photodiode. As it has been mentioned before, the photocurrent detected by the photodiode is very small. Therefore, the signal is amplified and conditioned in the amplification circuit shown in Fig. 2(b). The relationship between the final output signal V_o and the voltage input from the pre-amplification, V_{Iph} is given by Eq. (2).

$$V_o = \left(1 + \frac{R_3}{R_2}\right) V_{Iph} \tag{2}$$

In order to minimize the optical noise due to background measurement, an analog filtration; low pass filter and high pass filter were designed to bypass and filter the light signal. This was obtained by conducting simulation using Matlab-Simulink based on Eqs. (3) and (4) which were gained from the pre-amplification and the amplification circuits.

$$\frac{V_{Iph}(s)}{I_{ph}(s)} = \left[\frac{R_f}{1 + R_f C_f s} + \frac{R_1}{1 + R_1 C_1 s}\right] \tag{3}$$

$$\frac{V_o(s)}{V_{Iph}(s)} = \frac{(R_2 C_2 + R_3 C_2)s + 1}{R_2 C_2 s + 1} \tag{4}$$

The device was tested by performing measurements from bio-particles samples absorption detection in PDMS microfluidic channel. According to Beer's Law, absorbance (A) has the following equation

$$A = -\log_{10} \frac{I_1}{I_o} \tag{5}$$

where I_1 is transmitted light and I_0 is incident light. To enhance light absorption, the background signal was subtracted from the measurement signal. This background signal was measured by the photodiode when the LED is in OFF condition. Thus, the ratio of the difference between samples and background signals to the difference between reference and background signals provides the full absorbance that is shown by the following equation

$$A = -\log_{10} \frac{I_{sample} - I_{background}}{I_{reference} - I_{background}} \tag{6}$$

where I_{sample}, $I_{reference}$ and $I_{background}$ are the transmitted light intensity in voltages when the microfluidic chamber was filled with samples, no samples in the chamber and in ambient light. The schematic illustration of light absorption by biological molecules is shown in Fig. 3. First, UV light from the LED is emitted toward the microfluidic channel, and after the bio-particles pass through the chamber, the incident light is scattered and absorbed by the particles, reducing the intensity of the transmitted light [23]. The difference in light intensity level is evaluated by a photodiode located underneath the chamber and then recorded as a series of pulses.

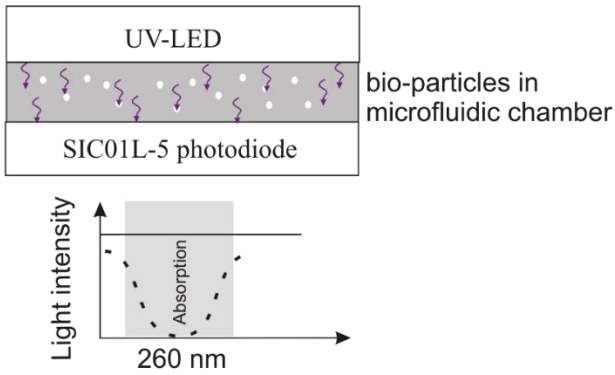


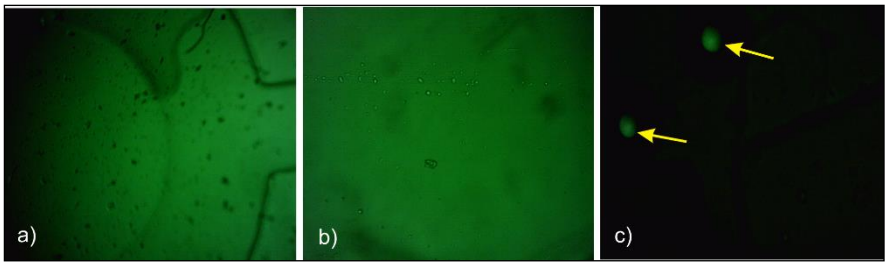
Fig. 3. Optical detection using absorbance method.

2.3. Testing procedures

Before conducting the test measurement, the system was initially optimized by varying the distance between UV-LED, chamber and SiC photodiode. The effect of the incident light emitted to the detector was investigated to determine an optimum optical path length of the device. The ball lens UV-LED substantially collimated the incident light in the range from 15 mm to 20 mm. The measurements were performed by varying the position of the UV-LED from the shortest to the longest range. The range was chosen based on the requirement of the LED to emit light with spot diameter within 1.5 mm to 2.0 mm.

The measurement of the bio-particle detection was performed by injecting the samples into the inlet of the microfluidic channel using a 50 µl glass syringe (Luer Tip. 50 µl, Hamilton) and 23 G needle. Four sample types including DI water, TE buffer, protein and DNA samples in TE buffer were tested for the absorption detection. The protein and DNA samples were obtained from Biosciences and Biotechnology department, UKM. The DNA was extracted from the human intestine; Caco-2 cell lines [24].

The data were accumulated over a period of 200 seconds with every 15 seconds. The light intensity was converted into output voltage signals and displayed using the oscilloscope. To establish the presence of DNA samples in the chamber, 10 µl of DNA sample was mixed with equal volumes of SYBR Green I (Sigma-Aldrich) which was diluted in the ratio of 1/10000 of PBS as a nucleic acid fluorescence stain. After the measurement was done, the sample was monitored under an optical microscope (BA400 Epi-Fluorescent, Motic) and the images were captured by an eyepiece camera (Dino-Lite AM423B Dino-Eye). This is to confirm the presence of absorbing bio-particle sample in the microfluidic chamber. Figures 4 showa three samples; DI water, protein and DNA from Caco2 that were observed under the fluorescent microscope. The stained DNA is clearly seen under the microscope as depicted in Fig. 4(c). While for DI water, Fig. 4(a) and protein, Fig. 4(b), there is only clear fluids sample that can be captured by the microscope since these two samples are not affected by SYBR Green I.



(a) DI water. (b) protein. (c) DNA from Caco2.

Fig. 4. Various samples captured from a fluorescent microscope.

3. Results and Discussion

Simulation results are given in Fig. 5 shows the low-pass filter characteristic of the pre-amplifier stage, which was designed to attenuate any high-frequency noise in the amplifier. The designed filter passed the modulation frequency at 1 kHz while high-frequency noise greater than 3180 Hz is attenuated. The photocurrent normally, in the rate of few nanoamperes, was converted to a proportional voltage and amplified at the gain of 120 dB. As the -3 dB bandwidth of the pre-amplifier circuit is equal to 3180 Hz, the rise time (τ_r=0. 35/f_{3dB}), as the time for the signal to rise from 10% to 90% of the steady state value, is calculated at 110 µs. The voltage signal was further amplified in the amplification stage at a gain of 20.7 dB. This phase incorporates an offset adjustment using -3 dB 155 Hz high pass filter (Fig. 6) in which low-frequency noise less than this frequency was blocked and again passed 1 kHz modulation frequency.

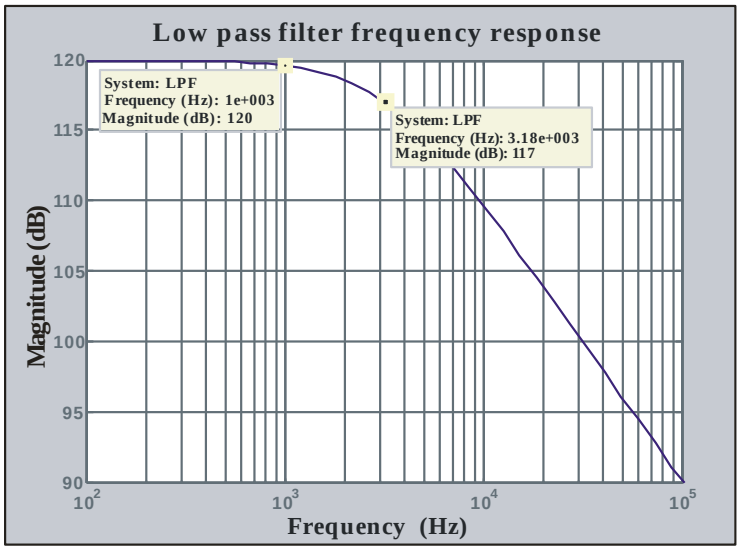


Fig. 5. Simulation results for pre-amplifier low-pass filter to attenuate high-frequency noise ≥ 3180 Hz.

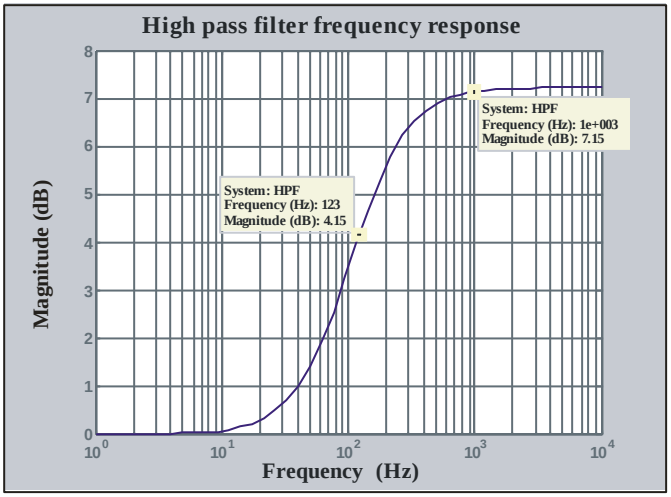


Fig. 6. Simulation results for amplifier high-pass filter to attenuate low-frequency noise ≤ 123 Hz.

An oscilloscope (LeCroy WaveSurfer 424 200 MHz) was used to capture and measure the signals from the LED and photodiode response under LED illumination. Figure 7 shows the quasi-CW input signal modulation frequency at 1.0829778 kHz generated by the LED driver on channel 2. The LED was pulsed ON for 500 µs at a duty cycle of 50.11 %. The voltage of the light illumination that is given by the amplitude in channel 2 is 4.684 V in order to drive the LED at 20 mA when the resistance parameter was at 237 Ω. The photodiode voltage response at similar frequencies, 1.0829778 kHz from the pre-amplifier circuit was depicted in channel 1. This measured voltage response is directly proportional to the light intensity due to the light emission from the UV LED. As the estimated rise time (τ_r) was given by 110 µs, the measured τ_r by the oscilloscope is 118.1371 µs that was a relatively high value for matching factor. This also indicates that the optical transducer has a rapid response for the detection process.

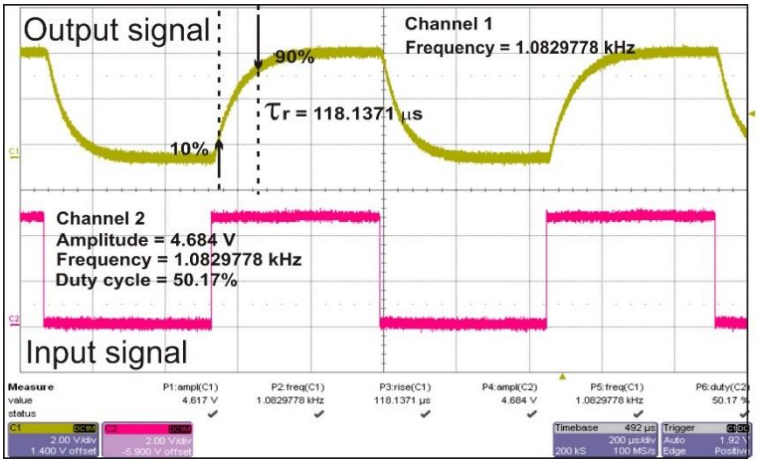


Fig. 7. UV LED and SiC photodiode signal responses.

Figure 8 shows the constructed bio-particle detection system, including fabricated PDMS based fluidic parts, and optical parts. The 4 mm diameter of the transparent chamber and the SEM image of the unenclosed chamber with 80 µm depth can be seen from Fig. 8. The total volume of the system is 30×30×30 mm^3. The vertical distance of the LED can be adjusted from 15 to 20 mm in order to find the optimal path length of the UV light.

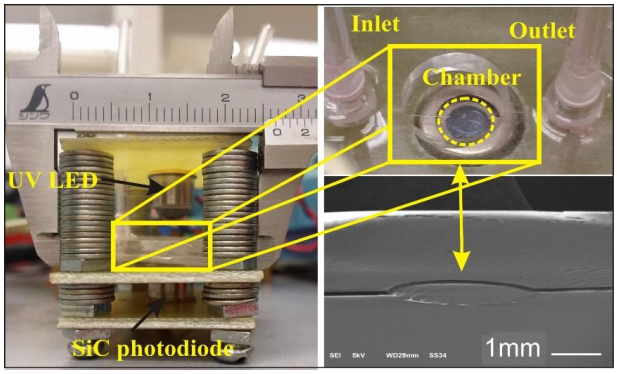


Fig. 8. Constructed of the bio-particle detector system.

The responses of the SiC photodiode voltages, as the light intensity voltage, to UV illumination and ambient light intensity at 260 nm for different light path lengths are illustrated in Fig. 9. The comparison of all these responses is to determine the optimal path length. During measurement, the UV-LED was positioned vertically with the distance from the photodiode between 15 to 20 mm. As it can be seen in Fig. 9, at a shorter length (15mm) the highest amount of UV light was received by the SiC photodiode. By increasing lengths to 16-19 mm, the voltage values showed a significant reduction in the quantity of voltage detected. In contrast, the SiC photodiode response to ambient light has a slight change in voltage values where the lowest value is related to 15 mm light path length. Therefore, based on this analysis result and in comparison with other light path lengths, the optimum light path length of 15 mm was chosen due to its highest light intensity voltage and lowest value of interference by ambient light.

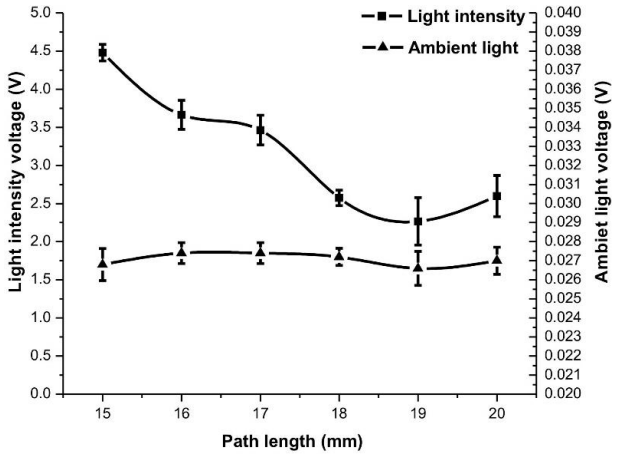


Fig. 9. Determination of the optimum optical path length.

The bar chart in Fig. 10 compares the amount of voltages received by SiC photodiode when the UV-LED is in ON and OFF condition in the lighted and dark room. It is shown that there is no significant difference in UV light intensity in both light and dark room, with the same voltage value of 4.5 V. Furthermore, as it is shown in Fig. 10, the SIC01L-5 photodiode is completely insensitive to the visible light >400 nm, which implies that the system can perform properly without any influence from the ambient light.

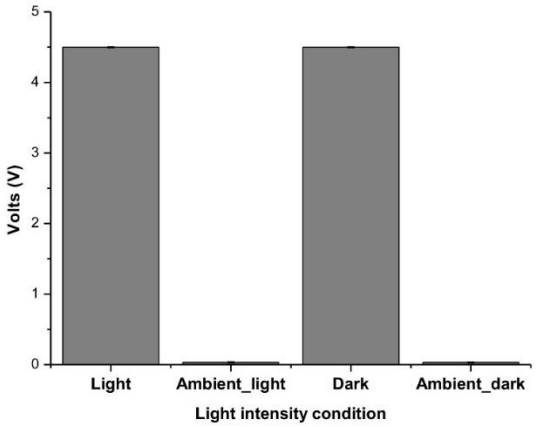


Fig. 10. SiC photodiode signal responses in ON and OFF condition.

The rise of 500 µs photodiode responses corresponding to the photocurrent is shown in Fig. 11. The photocurrent was obtained from the photodiode voltage response. The samples were injected into the chamber of the microfluidic using 50 µl glass syringe. As it is shown in Fig. 11, the measured photocurrent under the light intensity are compared for different samples in the chamber, i.e. DI water, TE buffer and DNA samples, as well as the PDMS microfluidic device without any medium of samples in the chamber.

Without any medium of samples in the microfluidic chamber (PDMS device result in Fig. 11, the photocurrent exhibits maximum values approximately at 2 µA. As it can be seen in the figure, both DI water and TE buffer samples reveal the same photocurrent value, showing no influence on the UV light intensity reaching the SiC photodiode, which is expectable since these mediums are non-absorbing UV light. The protein sample is also showing only slightly influence on the UV light since this medium absorbs light at 280 nm.

The effect of light absorption can be seen when the microfluidic chamber was injected with less than 10 µl of bio-particle DNA samples (extracted from Caco-2 cells) into the chamber, as the magnitude of the photocurrent is decreased. In this case, the bio-particle DNA sample absorbs the excitation light from the UV LED at 260 nm wavelength. As a result, this absorption led to the reduction of photocurrent value detected by SiC photodiode.

Absorbance calculation was performed based on the Lambert-Beer Law relationship in Eq. (6) and using the photocurrent measurement results depicted in Fig. 11. These various samples absorbance is shown in Fig. 12. Initially, the absorbance of DI water was measured, when the PDMS microfluidic device with no medium of samples in the chamber was used as the reference. As expected, DI

water did not absorb UV light as the absorbance is very low depicted in the analytical result (Fig. 12). The same result for the same reason is also shown in TE buffer and protein. The absorbance values for these non-absorbing mediums are remaining constant for a period of 200 sec. In contrast, the absorbance of the bio-particle DNA sample has a high absorbance on the average of 0.08 a.u since the DNA interacts with UV light through the absorption process.

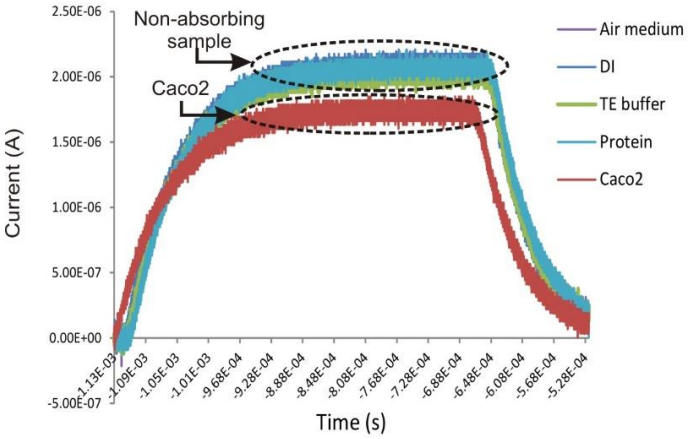


Fig. 11. Photocurrents response with samples.

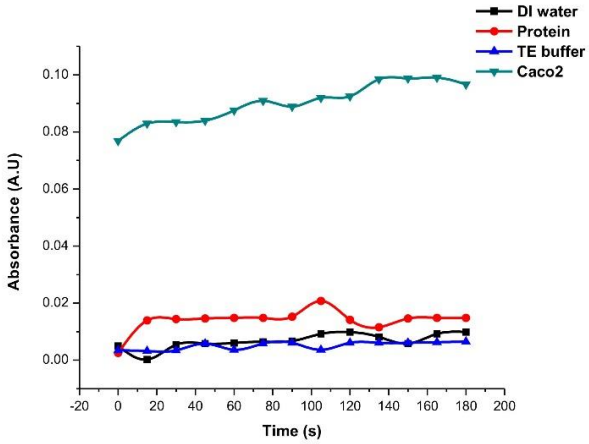


Fig. 12. The absorbance of various samples at 260 nm.

4. Conclusions

An optical transducer employing ultraviolet light emitting diode (UV LED) and photodiode has been developed for direct measurement of bio-particle samples. The transducer was integrated with PDMS based MEMS microfluidic system to enable high sensitive bio-particle detection.

- The UV light at 260 nm from the light source was successfully received by the photodiode within optimum optical pathlength. The response was in the form of a voltage with less noise and the transient response was measured.
- The significant parameter for the high-speed response of the optical transducer is given by the measured rise time. This was managed by performing the photodiode response under the illumination of the LED under ON and OFF condition.
- The sample absorption data collected from the device show less response for the non-absorbing samples. However, there was a significant interaction between the light and biological samples that made the light intensity reduced as the voltages were dropped and the absorbance analyzed is average at 0.08 a.u.
- With the integration of this vertical optical transducer with the PDMS microfluidic, it holds the potential to be utilized in medical diagnosis applications.

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