Development of Spectral Delayed Luminescence System for Whole Saliva Analysis: A Prototype Study
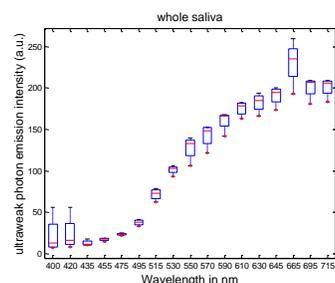
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Graphical abstract

Abstract

This paperwork presents the development of spectral delayed luminescence system for the measurement of whole saliva. Despite various reports of delayed luminescence and its correlation to various diseases, it has fail to highlight the specific cause of the occurrence suggesting it being more suitable as a screening tool utilizing saliva as non-invasive sampling medium. Therefore it is the aim of this paper to have the system critical performance tested before putting it to use at the field. The system prototype is tested for its response during room temperature operation and during spot cooling of the photomultiplier tube module. Delayed luminescence of saliva is compared over distilled water as a control. The spectral delayed luminescence of saliva is compared to that of the dimmed white light for functional testing purposes. Through experiments, the best operation for the system is at constant room temperature with light stimulation time determined at 150 ms. Being 98% water, the delayed luminescence of saliva is slightly greater than that of distilled water. Given light stimulation of wide spectrum from white LED, the delayed luminescence of saliva is accumulated between 495 nm and 530 nm. Results demonstrated that the system for spectral delayed luminescence of saliva is viable and capable for acquiring data for further studies.

Keywords: Delayed luminescence; spectral; prototype; whole saliva

Abstrak

Kertas kerja ini membentangkan pembangunan sistem spektra pendarkilau tertangguh bagi tujuan analisis air liur. Di sebalik pelbagai laporan korelasi pendarkilau tertangguh dengan pelbagai penyakit, punca cahaya yang dikeluarkan gagal dikemukakan mencadang kaedah ini lebih sesuai sebagai alat saringan menggunakan liur sebagai medium yang tidak invasive. Dengan ini, adalah menjadi matlamat laporan ini menguji prestasi secara kritikal sebelum menggunakannya di lapangan. Prototaip sistem ini telah diuji tindakbalasnya ketika beroperasi dalam persekitaran suhu bilik dan ketika modul tiub pengganda cahaya disejukkan. Pendarkilau tertangguh air liur telah dibandingkan dengan air suling sebagai elemen kawalan. Spektra pendarkilau tertangguh daripada air liur dibandingkan dengan spektra pendarkilau tertangguh cahaya putih bagi tujuan pengujian fungsi. Melalui ujikaji, sistem berfungsi terbaik pada suhu bilik malar dengan tempoh stimulasi cahaya ditentukan pada 150 ms. Terdiri daripada 98% air, perdarkilau tertangguh liur adalah lebih besar sedikit daripada air suling. Distimulasikan dengan cahaya putih LED berspektrum luas, perdarkilau tertangguh liur bertumpu antara 495 nm dan 530 nm. Keputusan menunjukkan sistem untuk spektra pendarkilau tertangguh adalah berdaya fungsi dan berkemampuan untuk pengumpulan data dalam kajian selanjutnya.

Kata kunci: Perdarkilau tertangguh; spectra; prototaip; air liur asal

■1.0 INTRODUCTION

Saliva is an important fluid in the body. Being 98% water, it contains enzymes the body needs to digest food and antimicrobial peptides including defensins for the purpose of innate host defense, DNA, RNA, proteins, immunoglobulin, metabolites, bacteria and cellular materials [1, 2]. The levels of enzyme, peptides, and defensins produced by the salivary glands can be affected by oral diseases or other related diseases. Therefore, like blood serum, saliva can be a useful indicator of various conditions for risk assessment, salivary diagnosis and therapeutic strategies [1, 2].

There are various optical methods to analyze specific content in saliva. UV spectrophotometry is used to detect DNA in saliva [3], chemiluminescence is employed to monitor continuously roxithromycin levels in saliva via flow injection method [4], spectral analysis of saliva to detect lung cancer marker uses the surface enhanced Raman spectroscopy method [5], infrared attenuated total reflection spectroscopy is utilized to detect drugs by saliva spectral analysis [6] and testosterone levels are measured

in patients with androgenic disorders using the saliva luminescence immunoassay [7]. Each and every method measures different components of saliva and have their own unique range of spectrum which differs from each other. For example, saliva luminescence immunoassay accesses specific hormone in saliva and in order to achieve that, specific antibodies need to be added to the saliva in the measurement process. Delayed luminescence too owns its unique method in measurement of saliva or any other biological tissues and fluid. It measures the luminescence of samples after being induced with light and hence it is name delayed luminescence and it is carried out without any addition of reagent since it is not the objective of this method to observe any biochemical components of samples.

Recently, delayed luminescence has been utilized as method to research the dynamics of biological systems via means of its response to light induction. It is known that stressed biological systems exhibits high photo-induced delayed luminescence readings as compared to the norm [8]. Spectral characterization of delayed luminescence too has been of interest in understanding the nature of delayed luminescence of organisms. Initially measurements of delayed luminescence are focused on plants and microorganisms [9-12]. However, there has been a noticeable trend in applying delayed luminescence for discrimination of normal and diseased conditions in humans. This include lung cancer tissues[13], cancer skin cells in-vitro[14, 15] and in-vivo [16, 17], tumor cells [18, 19], blood serum for correlation with diabetics [20] and leukemic [21]. However, delayed luminescence does not indicate specific disease. Being that the case, it is proposed that the delayed luminescence method be a screening tool for indication of acute and chronic diseased state of a person as a whole.

A screening tool is most appropriate if sampling from the subject is done non-invasively. Saliva is chosen to be the most suitable candidate. Furthermore, spectral delayed luminescence of whole saliva has not been investigated. In order to achieve this objective, a prototype system for measurement of spectral delayed luminescence for whole saliva is developed. Before the actual measurements of saliva for normal and diseased subject are carried out and reported in another experiment, it is essential that few performances of the developed prototype are tested. This paper describes the how the prototype performs when the core sensor, the photomultiplier (PMT), is both cooled and operating at room temperature. It is also essential that this prototype can differentiate between water and saliva, in which 98% of it is also water. A benchmark measurement of the spectral delayed luminescence of saliva against the wide spectrum of white light will provide a high level confidence that the prototype is functioning and not just giving arbitrary measurements. Calibrations of the prototype for future measurements will be based on the results presented. Comparisons of stimulation with lights other than white such as UV, infrared, red, blue or green are reported in another journal sent for peer-review elsewhere.

Cooling of the PMT has been reported to reduce the internal thermal noise and a common measure taken by delayed luminescence authors is by cooling the PMT itself below -20°C [12, 20, 21]. Cooling can be achieved by the peltier thermoelectric module [22, 23] or by circulating cooled liquid or gas [18, 24] around the PMT. There are also studies measuring the delayed luminescence with PMT at ambient room temperature of 20°C [25, 26]. External factor have been reported to have influenced the delayed luminescence characteristics [27] and therefore the influence of temperature conditions and illumination time in relation to delayed luminescence response needs to be studied. The following section 2.0 describes the methodology flow in developing and experimental testing of the prototype system. In addition, section 3.0 presents the results of the experimental testing. The result is used to determine the parameter setting of the final

working of the prototype. A conclusion that summarizes the whole study and proposes future work is made at section 4.0.

■2.0 METHODS AND EXPERIMENTS

Setup for every delayed luminescence is unique and customized for every different application. Most combined standard available modules and arrange according to their study. This study maintains low cost development profile and therefore the photon counting module is developed based on previous system with modifications [28].

Figure 1 illustrates the flow of task in achieving the expected outcomes of this study. Hardware arrangements are design for measuring spectral delayed luminescence from filter paper strip placed inside UV cuvette. The arrangements are made so to enable the experiments to follow. Modifications in [28] are made to customized for measurement of single photon using the dedicated sensor, the photomultiplier tube module. Completing the hardware setup, instrumentation and acquisition, the optical calibration obtained by calculation prior to the data collection for the following experiments as illustrated.

The first experiment is to determine the optimal temperature condition and the minimal stimulation time required for photo-excitation of the saliva sample. The following experiment examines the functional of the system by measuring a few sample material and liquid. The final experiment then measures the spectral delayed luminescence of whole saliva, which is the main purpose of this study.
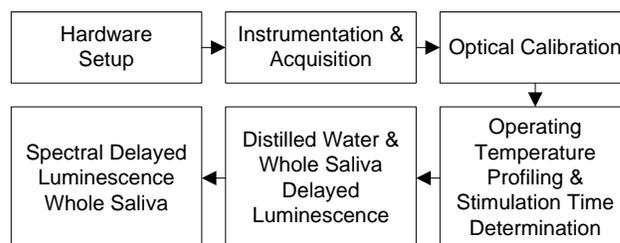
Figure 1 Methodology

2.1 Hardware Setup

Figure 2 illustrates the physical arrangement of the hardware in realizing this system. The sample holder, an ultraviolet cuvette and the sample chamber is located in front of the PMT window. In between the PMT and the sample is the optical filter disk which houses 17 long-pass cutoff optical filters arranged around its circumference. These set of 17 optical filters are manufactured by Edmund optics with the parts numbers GG-400, GG-420, GG-435, GG-455, GG-475, GG-495, OG-515, OG-530, OG-550, OG-570, OG-590, RG-610, RG-630, RG-645, RG-665, RG-695 and RG-715. These combination long-pass cutoff filters are used to produce a spectral delayed luminescence system of 16 wavelength ranging from 400 nm to 715 nm. An additional all-pass opening is provided in the optical disk if no filter is to be used. The optical filters disk is controlled by a 360 degrees servo motor. A microcontroller controls the pulse-width-modulation to the servo motor with duty-cycles programmed with respective to the location of each optical filters on the disk.

Peltier thermoelectric module cooler is attached to the metal body of the PMT to provide spot cooling. Cooled PMT reduces ambient dark current. Heat sink and an exhaust fan is used to remove the heat from the hot-side of the peltier module. With room

temperature at 25 degrees Celsius, a spot cooling temperature of -40 degrees Celsius can be accomplished. However, in order to avoid condensation from humid air, a room environment with relative humidity of 40% is desirable.

A shutter with electronic control is assembled between the sample chamber and the optical disk so as to prevent the stimulating light entering the PMT when the sample is being illuminated. The high intensity stimulating light may damaged the highly sensitive PMT.

Enclosed in a dark box made of acrylic and sealed with black epoxy, light leakage test are carried out by measuring the light intensity in the dark chamber using the developed acquisition system.
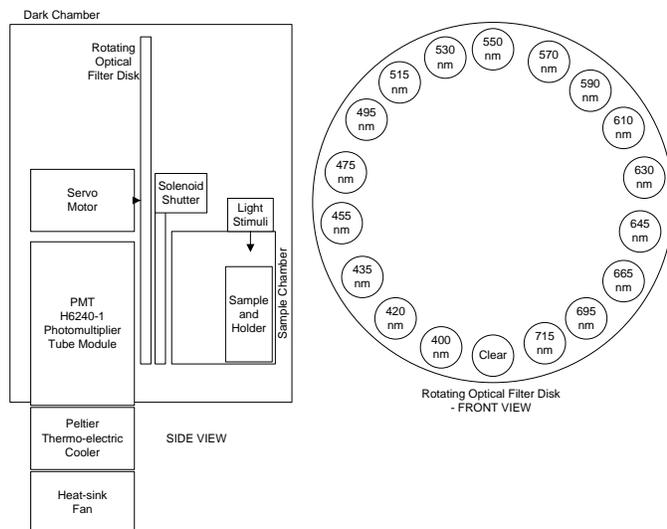

Figure 2 Hardware setup of spectral photon-counting system

2.2 Instrumentation and Acquisition

Development of spectral delayed luminescence acquisition system is described in Figure 3. The photon sensor employed is a low noise photomultiplier tube (PMT) module with built-in power supply, H6240-01 from Hamamatsu. With optimum factory adjustment of signal-to-noise ratio, the PMT has an optical transmission wavelength ranging from 200 nm to 700 nm. The module with side-on optical window is power externally by +5 volts source and generates an output pulse when a single photon is detected. Being compact and lightweight, it enables construction of a low power and portable spectral delayed luminescence acquisition system. The intensity of the detected ultra-weak photon emission is indicated by the count rate of pulses from the PMT which is then registered by the four 4-bit high speed logic binary counters in cascade enabling up to 16-bit resolution.

The core controller of the system is the PIC18F4550 Microchip 8-bit microcontroller. The microcontroller is programmed to receive control settings from the PC software via the dual channel parallel USB interface chip FT2232. The microcontroller will then turn the optical disk to the selected wavelength, turn on the light stimuli for the set duration, and start latching data from the four 4-bit counters and sending the data back to the PC for recording. Time interval between each sampling too is controlled by the microcontroller according to the value set by the user on the PC software.
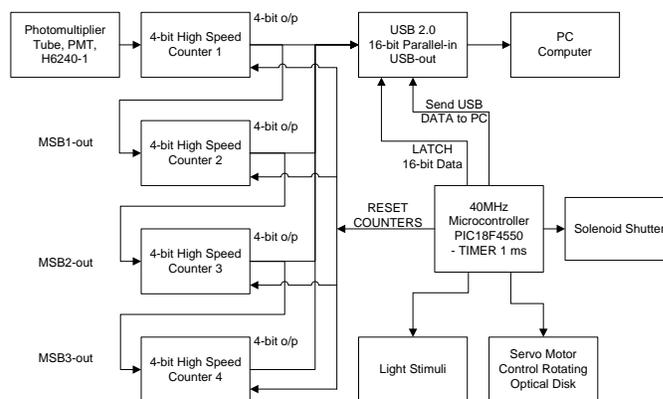

Figure 3 Electronic acquisition system setup

The software on the PC is developed using the Microsoft Visual C++ 6.0. Before initiating photon counting acquisition, settings are made for every new record. The parameters setting are sent to the microcontroller via the USB driver and the software waits for acknowledgement before acquiring, sorting and saving the photon count data into the respective wavelength channel.
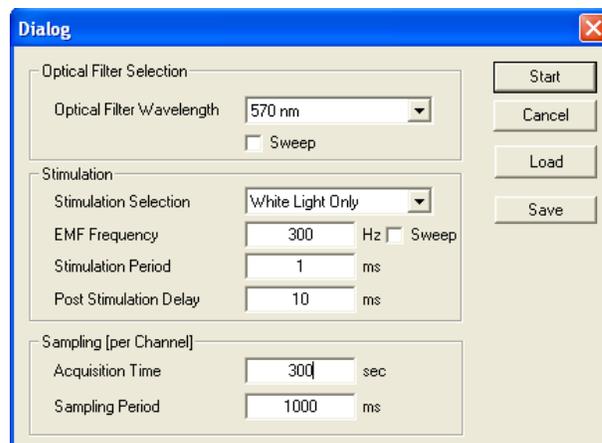

Figure 4 Software setting for photon-counting system

2.3 Optical Calibration Setup

Transmittance data of each long-pass cutoff optical filter are obtained from the Edmund Optics manufacturer's datasheet. The transmittance data of the PMT is obtained from the Hamamatsu manufacturer's datasheet. Figure 5 shows the transmittance of each optical filters employed. The average wavelength difference between each optical filter is approximately 20 nm.
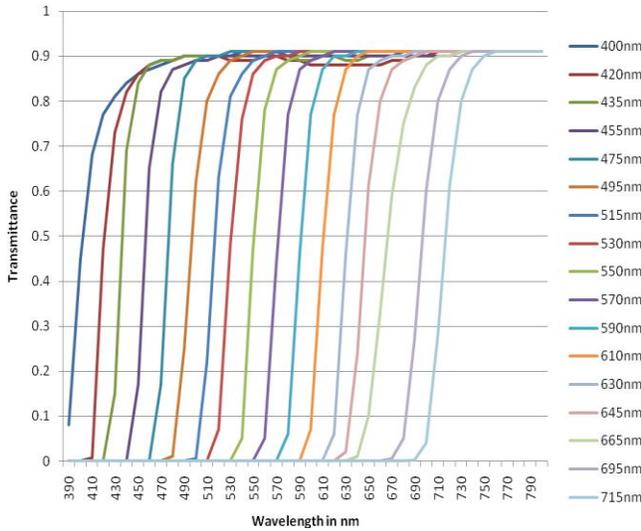
Figure 5 Long-pass cutoff optical filters characteristics

The transmission characteristics P_{ij} between the ith and j^{th} successive colored glass filters and the PMT is expressed as follows in equation 1

$$P_{ij} = (N_i - N_j) / d\lambda \int Sc(\lambda)[F_i(\lambda)-F_j(\lambda)]…………..(1)$$

Where $N_i - N_j$ is the subtraction of the count rate and $F_i(\lambda) - F_j(\lambda)$ is the subtraction of the transmission curve of the ith and j^{th} successive filters, $Sc(\lambda)$ express the transmission of the photomultiplier [22].

The band-pass transmittance characteristic between each optical filter is calculated by the subtraction of the transmission curve of the current and the adjacent successive filters as in Equation 1. The resulting subtraction of the calculated band-pass transmittance between each optical filters employed are as plotted in Figure 6. Topmost of the figure shows the transmittance characteristics of the PMT obtained from the Hamamatsu manufacturer's datasheet.
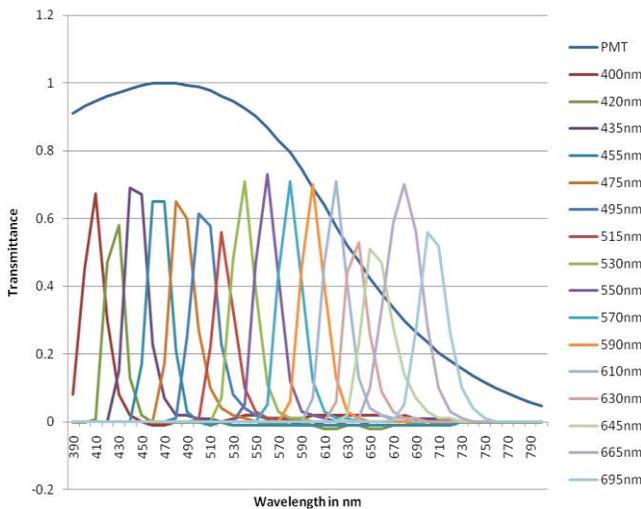

Figure 6 PMT transmission characteristics and band-pass optical filters response

The final total transmittance of both the PMT and band-pass optical of each wavelength range are multiplied and the results are illustrated in Figure 7. All spectral delayed luminescence measurements will be calculated for its final photon count values base on the total transmittance.
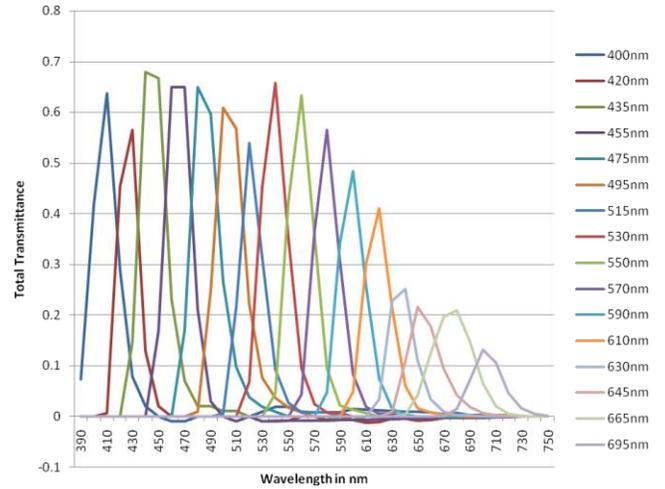

Figure 7 Combined transmission of photomultiplier and respective optical filter

The calculated transmission characteristic values, P_{ij}, for each of the optical filter are as in Table 1. Respective N_i and N_j are obtained from the photon counting system described earlier.

Table 1 Optical filters calculated calibration

Wavelength, nm	Calibration Values
400	15.15842
420	11.71269
435	18.50677
455	17.08873
475	18.48467
495	17.95435
515	12.164
530	16.67409
550	15.99206
570	13.9225
590	12.25337
610	10.65066
630	6.748974
645	6.789255
665	8.199324
695	3.948872

Calibration spectrum of the light stimulus is obtained for a dimmed white LED. The white LED is powered by 5.0 volts and connected in series with a 2.0 Mega ohm resistor. With the white LED on throughout the recording, the spectral transmission of the LED is registered.

2.4 Operating Temperature Profiling Against Stimulation Time

Operational temperature profiling with varying stimulation time is to be portrayed so that the optimal operating temperature and stimulation time can be determined for the subsequent experiments.

With the setup as described in Figure 2, measurements of delayed luminescence will be recorded for varying stimulation time beginning with 50 ms to 1000 ms with intervals of 50 ms. Each measurement of each stimulation time is repeated 20 times. These procedures are carried out for both operation temperatures, that is, during room temperature of 24°C and during PMT spot cooling. In order to avoid condensation on the spot cooled PMT, the room temperature must be 24°C and the relative humidity must be within 40% RH.

The light stimulus employed for this study is sourced from a bright white LED. However, the delayed luminescence signal acquired includes both the afterglow of the white LED and the ultra-weak photon reemission of the whole saliva. In order to ensure high signal intensity of the signal over the stimuli, the white LED is dimmed to its minimum, detectable by the PMT, by increasing the resistance in series with it. Through several iterations, the series resistor selected is 2.0 M Ohms power by 5.0 volts. This selection produces white light with the lowest intensity not visible to the eye even in the dark but is able to be detected by the highly sensitive PMT.

Delayed luminescence at this phase is done without any saliva sample nor holding media except for the dark chamber itself. The photon counts during the delayed luminescence each are recorded for 30 seconds with 1 second interval.

2.5 Delayed Luminescence Distilled Water and Whole Saliva

A dry filter paper strip is placed inside the UV cuvette and measured for its delayed luminescence. This is followed by measurement of saliva wetted on filter paper strip. Saliva is obtained from a single volunteer aged in his thirties. He is seated relax for 30 minutes prior to sampling. Filter paper strip is placed on his dorsal tongue to wet it with his saliva. No further processing of the whole saliva is carried out. Measurement of the whole saliva is done as it is. For reference, the delayed luminescence of distilled water is also measured. Measurements are recorded for 60 seconds with 1 second interval and are repeated twice for each sample.

2.6 Spectral Delayed Luminescence Whole Saliva

Prior to measurement of delayed luminescence of whole saliva, the spectral delayed luminescence characteristics of the white light stimulus are to be charted beforehand.

During stimulation with an empty sample chamber, the dimmed white LED is powered on for a brief time period of 150 ms. The delayed luminescence is then recorded for 60 seconds with sampling intervals of 1 second. This sequence is repeated for each optical filter selection. The spectral delayed luminescence of the dimmed white LED is recorded 5 times.

The same procedure is then applied for whole saliva on filter paper strip. The saliva is obtained from the same volunteer mentioned previously.

■3.0 RESULTS AND DISCUSSION

Delayed luminescence data are retrieved, processed, plotted and analyzed using MATLAB 6.1. The following subsection presents the operational temperature response with varying photo-stimulation time. This is followed by section 3.2 which conveys the raw signal response of the developed system using three different materials. The spectral delayed luminescence of whole saliva which is the main objective of the study is presented in section 3.3 with spectral of the dimmed white LED as a reference.

3.1   Comparison Delayed Luminescence Temperature Influence on Stimulation Time

Mean value of slopes for varying stimulation time tested for room temperature operation and spot cooling of the PMT is graphed in Figure 8. For both room temperature and PMT cooled operation, delayed luminescence slope decreases with increased stimulation time. It is, however, observed in the graph that the room temperature measurement is more consistent. It is a requirement that the desired stimulation time is the minimum and at the same time the slope is lesser than 1. From the figure it is clear that the stimulation time at 150 ms fulfill the requirement and both room temperature and PMT cooled operation at this point correlates.
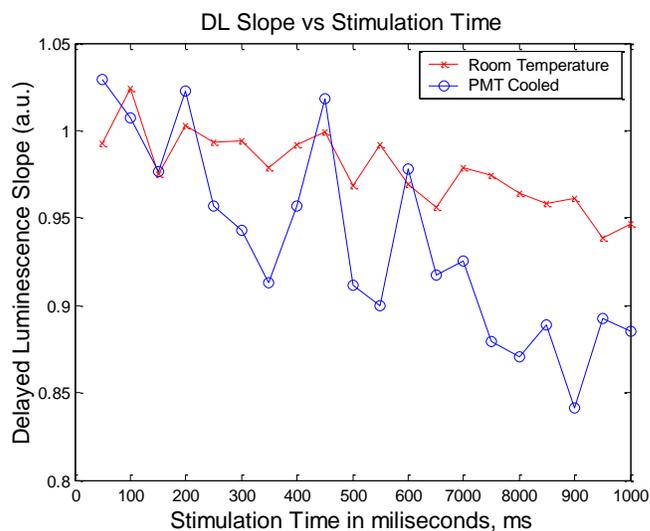
Figure 8 Mean value of slopes of decaying delayed luminescence for a range of stimulation time

In order to verify the stability of operation for both conditions, standard deviation of 20 measurements of each condition is plotted in Figure 9. Throughout the varying stimulation time, room temperature operation exhibits lower mean standard deviation of ±0.0527 compare to the PMT cooled which mean standard deviation is ±0.1005. It is obvious from the plot that room temperature operation is more unwavering throughout the varying stimulation time compare to the PMT cooled. It is therefore decided that the rest of the experiments are to be operated at room temperature of 24°C as mentioned earlier.
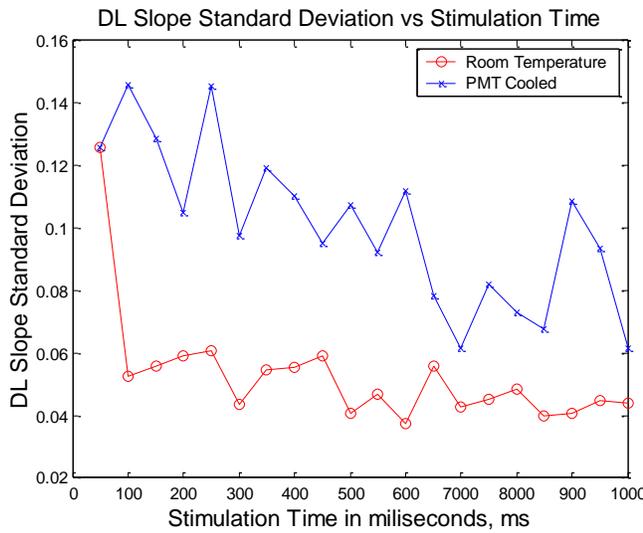
Figure 9 Standard deviation of delayed luminescence slopes for varying stimulation time

3.2 Delayed Luminescence Distilled Water and Whole Saliva

Three delayed luminescence signals of filter paper strips, distilled water and whole saliva is as seen in Figure 10. Each sample is measured twice and is labeled according the legend provided. Since saliva is 98% water, the best control chosen for this experimental analysis is pure or distilled water. Filter paper strips exhibits the least delayed luminescence photon counts. Delayed luminescence of distilled water on filter paper strip demonstrates high photon re-emissions than filter paper alone. Filter paper strip wet with saliva shows the highest delayed luminescence among the three. Repeated measurement of the three samples indicates high reproducibility even though there is observable subtle variation between the two saliva measurements.
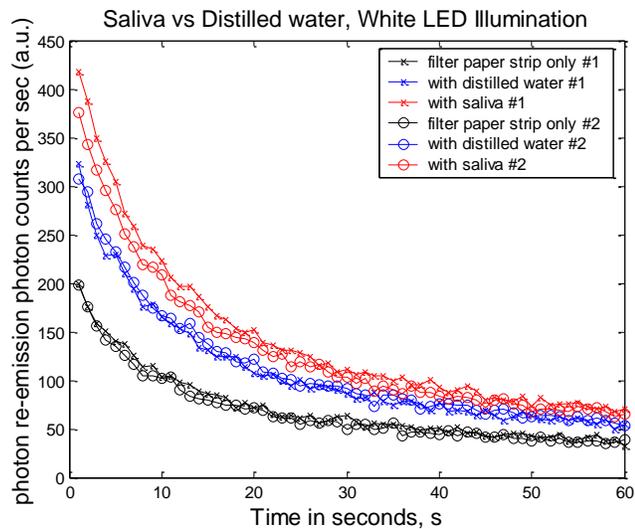

Figure 10 Delayed luminescence of distilled water (blue line x) and whole saliva (red line o) with white LED illumination

3.3 Spectral Delayed Luminescence Whole Saliva and Dimmed LED

Five repeated transmission measurement of dimmed white LED for every optical filter wavelength is as depicted in Figure 11. For each optical filter, the photon counts over 60 seconds are summed yielding value for the integrated photon counts. It is obvious that the median for every long-pass cutoff optical filter shows increasing values starting from the lowest wavelength 400 nm. From the box plot, it can be noted that the standard deviation for certain wavelength is fairly large.
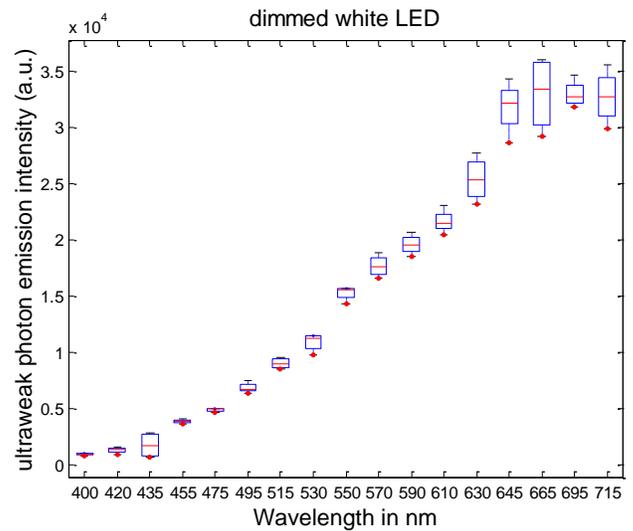

Figure 11 Spectral transmission of dimmed white LED for each cut-off optical filter

Spectral measurement is obtained by calculation from the spectral transmission data using equation 1 as described earlier. Continuously lit dimmed white LED spectrum is as depicted in Figure 12. Peaks can be observed at 435 nm and 630 nm. These peaks most probably are not of the white LED origin but more rather due to the fluctuation of the PMT measurement. This can be confirmed by the large standard deviation values of Figure 11 at wavelength 435 nm and 630 nm respectively.
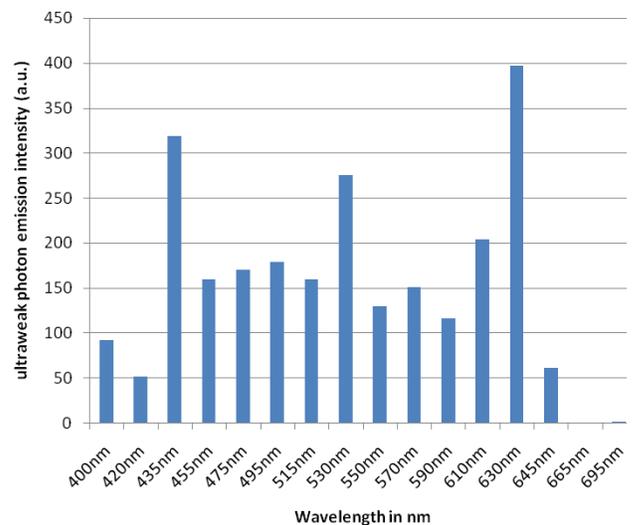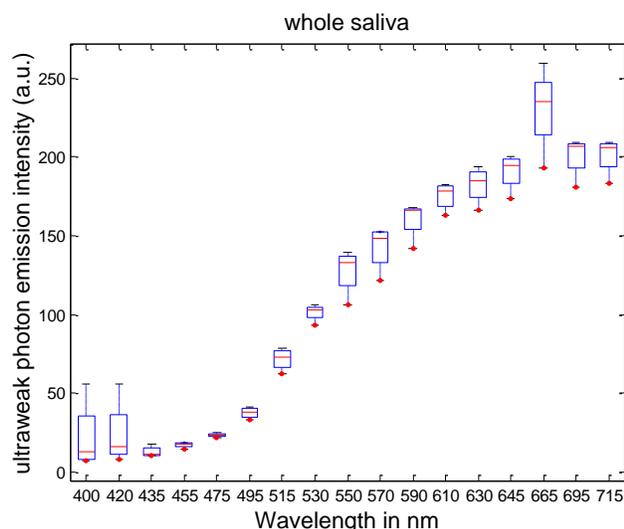

Figure 12 Spectrum of dimmed white LED

Comparing to the transmission plot of dimmed white LED to the transmission of whole saliva in Figure 13, the mean measurement of dimmed white LED is more linear than the mean measurement of the whole saliva. A steep increase of delayed luminescence between wavelength 495 nm and 530 nm can be observed in Figure 13.

Figure 13 Photo-induced ultra weak photon re-emission of whole saliva for each optical filter

Figure 14 is the resulting spectrum obtained from whole saliva. It is obvious that the spectral delayed luminescence of whole saliva concentrate to the lower domain of the spectrum ranging between 475 nm and 530 nm. Peak at 645 nm may also be the resulting high fluctuation during measurement and this is confirmed by observing wavelength 665 nm of Figure 13.
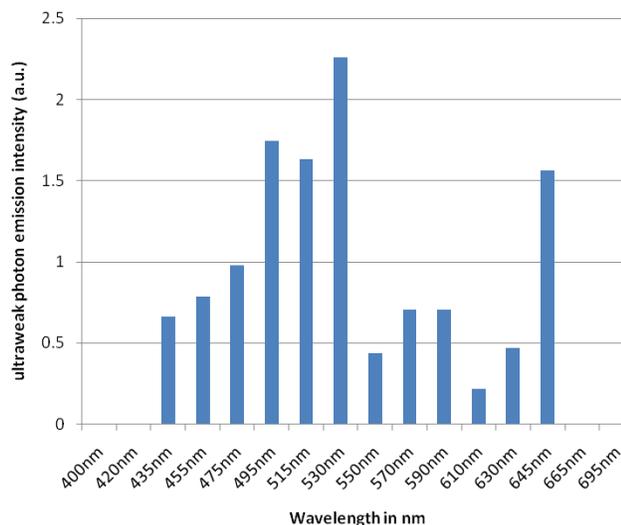
Figure 14 Spectral delayed luminescence of whole saliva

3.4 Discussions

Photon re-emission from light excitation, other than delayed luminescence, may be from phosphorescence, delayed fluorescence, or chemical luminescence or combinations of the mentioned. Besides that, the light re-emission may also be influenced by thermal effect [29]. Results from this study (Figure 8 and 9) shows contrary to the reviews which state that when the PMT is cooled, ambient dark noise is reduced to its minimum thereby increasing the signal to noise ratio of the measurement of delayed luminescence. However, the thermoelectric peltier used might contribute to the unstable fluctuation during repeated measurements. Even though the PMT is spot cooled, high electric currents flow through the thermoelectric module inducing electromagnetic fields (EMF) into the functioning mechanism of the PMT. Weak EMF may also leak into the PMT even though it is shielded and this reflects the high standard deviation measurement of delayed luminescence during spot cooling compared to when the PMT operates while the thermoelectric module is powered down.

Oxygen has been shown to have significant quenching effect on delayed luminescence [21]. It is suggested that deoxygenation process is carried out prior to measurement of luminescence [30]. Water, which contains oxygen, also does have quenching effects. Measuring seeds ability to store water using delayed luminescence has been used to identify the viability of seeds [31]. In another study, delayed luminescence of liquid serum is much lower than dried serum [29]. This effect confirms the result in figure 10. It is expected that if the saliva on the filter paper is dried before measurement, it might show higher delayed luminescence than wet. Drying of the saliva sample for future studies also eliminates the effect of water quenching which may not reflect the objective of identifying various disease conditions.

Delayed luminescence of biological material is said to be coherent and follows the law of hyperbolic decay rather than the exponential thermal luminescence decay. Laser light is known to be coherent, and even though white LED light is less coherent than laser light, repeated measurement of optical transmission exhibits relatively low standard deviation throughout spectral wavelength up to 610 nm (Figure 11). Wavelength 630 nm and above most probably reflect the thermal effect from the LED itself. Considering this, delayed luminescence of whole saliva for wavelength 435 nm through to 495 nm is relatively low (Figure 13), and this might be indicating coherent whole saliva spectral delayed luminescence response at the mentioned wavelength. On the other side, high standard deviation of repeated delayed luminescence measurement may indicate non-coherent. This suggest for future work so that these hypothesis can be confirmed by more future experiments.

■ 4.0 CONCLUSION

Spectral delayed luminescence of whole saliva is investigated using a custom developed and assembled system. The system includes a highly sensitive photomultiplier, optical filters disk and a control system using microcontrollers, high speed pulse counters and a dual channel USB interface. Data acquisition parameters and control settings of the optical disk can be done using software which is developed to interface to the hardware system via the USB port. Dimmed white LED is employed as the illumination source. The PMT is better off operated at room temperature than when it is spot-cooled using the peltier thermoelectric module attached to it chassis. In addition, delayed luminescence of distilled water almost closely resembles the whole saliva, therefore future measurement will study how air-dried whole saliva affect the measurements. Spectral delayed luminescence of the dimmed white LED without any sample served as a reference. When whole saliva is induced with white LED, the sample exhibits spectral delayed luminescence with high intensity at wavelength less than 550 nm. Future study of coherent and its relation to standard deviation of repeated spectral measurement may provide more features for the delayed luminescence of saliva. Furthermore, this research has shown the

spectral delayed luminescence characterization of whole saliva via the simplicity of sample preparation. It is expected to be a potential candidate as a fast screening tool to identify the health status of individuals.

Acknowledgement

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