

SPECTRAL DELAYED LUMINESCENCE SYSTEM FOR HUMAN SALIVA
SCREENING

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To my beloved parents, wife and children for their great patience

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ABSTRACT

Delayed luminescence (DL) is a measurement method utilizing the decay of photon counts recorded, after one second or more, after a sample is exposed to a stimulating light source. DL has been studied on various human samples including blood serum, lung cells, cancerous and tumor cells, and the skin, except saliva. Previous studies have cross-correlated the DL of respective human samples with a range of diseases comprising of diabetes, leukemia, lung cancer, and tumor. However, recent studies have shown that DL is not cell type specific due to the unknown mechanism of the photon emission. Hence, this method is not viable for the diagnosis of complex diseases but it is proposed for non-invasive disease screening. Saliva, which can be obtained non-invasively, was anticipated for the screening of diseases using the DL method. Therefore, the aim of this study is to identify the potential application for the screening of diseases using the spectral delayed luminescence (SDL) of saliva. In order to achieve this, a prototype DL system was developed. The prototype DL system was then tested with and without the cooling of the photomultiplier tube (PMT). Illumination of the sample in the DL system was then tested with the ultraviolet (UV) light emitting diode (LED) which was then compared against the white LED. DL without the PMT cooling system, shows lower photon count deviations when compared to the PMT cooled at -8°C for stimulation time ranging from 50 to 950 ms. In addition, the UV LED stimulation showed higher DL photon counts compared to white LED stimulation. Optimal stimulation time was then iterated for the SDL measurements of saliva and tongue swab, and it was found that 0.5 s is the optimal stimulation time. The first set of SDL measurements of saliva and tongue swabs were model fit into eight classes of mouth conditions. The classification performances of the respective models were then tested against the second data set of SDL measurements from the same respective sample. Results show that the most significant application of the SDL system is in detecting conditions related to mouth sores. This significance is dependent on both the SDL measurements of saliva and tongue swab with detection performance of 100 % sensitivity, 85 % specificity, and 93 % non-error rate.

ABSTRAK

Pendarkilau tertangguh (DL) adalah suatu kaedah pengukuran penyusutan kiraan foton, yang dicerap selepas satu saat atau lebih, setelah didedahkan kepada rangsangan sumber cahaya. DL telah dikaji menggunakan pelbagai sampel manusia termasuk serum darah, sel-sel paru-paru, sel-sel kanser dan ketumbuhan dan tiada kajian DL yang menggunakan air liur. Kajian-kajian yang lepas mengkaji hubungkait DL dengan sampel manusia untuk pelbagai penyakit termasuk kencing manis, leukemia, kanser paru-paru, dan ketumbuhan. Walaubagaimanapun, kajian yang lepas telah menunjukkan bahawa DL bukan khusus kepada mana-mana sel kerana tidak diketahui mekanisma pancaran cahaya tersebut. Dengan itu, kaedah ini tidak sesuai bagi diagnosis penyakit kompleks tetapi dicadangkan untuk saringan penyakit dengan cara bukan invasif. Air liur, yang boleh diperolehi dengan cara bukan invasif, dicadangkan untuk saringan penyakit dengan menggunakan kaedah DL. Oleh itu, kajian ini adalah bertujuan untuk mengenalpasti potensi aplikasi saringan penyakit dengan menggunakan spektra pendarkilau tertangguh (SDL) daripada air liur. Bagi mencapai tujuan ini, prototaip DL telah dibangunkan. Prototaip DL ini telah diuji bacaannya dengan dan tanpa sistem penyejukan tiub pengganda cahaya (PMT). Pencahayaan sampel dalam sistem DL ini kemudiannya diuji dengan diode pemancar cahaya (LED) sinar ungu (UV) dan dibandingkan dengan LED bercahaya putih. DL tanpa penyejukan PMT menunjukkan sisihan piawai kiraan foton yang lebih rendah dibandingkan dengan PMT yang disejukkan pada suhu -8°C bagi tempoh rangsangan cahaya berjulat antara 50 ms dan 950 ms. Rangsangan LED UV menunjukkan kiraan foton DL yang lebih tinggi dibandingkan dengan rangsangan cahaya LED berwarna putih. Tempoh rangsangan optimum kemudian telah dilelarkan bagi pengukuran air liur dan sapuan lidah, dan hasil menunjukkan tempoh yang terbaik adalah 0.5 s. Set pengukuran pertama SDL daripada air liur dan sapuan lidah telah digunakan untuk penentuan model berdasarkan kepada lapan kelas keadaan mulut. Prestasi klasifikasi bagi model-model yang berkaitan kemudiannya telah diuji terhadap set pengukuran kedua daripada set sampel yang sama. Hasil kajian menunjukkan bahawa aplikasi sistem SDL adalah paling bermakna untuk pengesanan keadaan yang berkaitan dengan sakit mulut. Ini adalah bergantung kepada kombinasi pengukuran SDL air liur dan sapuan lidah, yang mana prestasi pengesanan yang diperolehi adalah 100 % sensitiviti, kekhususan 85 % dan kadar tanpa-ralat 93 %.

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LIST OF ABBREVIATIONS

3D	-	3 dimension
ANOVA	-	Analysis of Variance
BSA	-	Bovine Serum Albumin
CCD	-	Charge-Coupled Device
COM	-	Communication
DL	-	Delayed Luminescence
DNA	-	Deoxyribonucleic Acid
EMF	-	Electromagnetic Field
ER	-	Error Rate
HF	-	High Frequency
HR	-	Heart Rate
IC	-	Integrated Circuit
IgA	-	Immunoglobulin A
LDA	-	Linear Discriminant Analysis
LED	-	Light Emitting Diode
LF	-	Low Frequency
LV	-	Latent Variable
MATLAB	-	Matrix Laboratory
NER	-	Non-Error Rate
PC	-	Personal Computer

PCA	-	Principal Component Analysis
PCB	-	Printed Circuit Board
PCI	-	Peripheral Component Interconnect
PLS	-	Partial Least Square
PLSDA	-	Partial Least Square Discriminant Analysis
PMT	-	Photomultiplier Tube
RH	-	Relative Humidity
RNA	-	Ribonucleic Acid
ROS	-	Reactive Oxygen Species
SDL	-	Spectral Delayed Luminescence
TTL	-	Transistor-Transistor Logic
UPE	-	Ultraweak Photon Emission
USB	-	Universal Serial Bus
UV	-	Ultraviolet

LIST OF SYMBOLS

cps	-	Counts per seconds
dB	-	Decibel
°C	-	Degrees Celsius
Hz	-	Hertz
Mb	-	Mega bit
MHz	-	Mega Hertz
μL	-	microlitre
μs	-	microseconds
ms	-	milliseconds
nm	-	nanometer
ns	-	nanoseconds
Ω	-	Ohms
t	-	time
V	-	Volts
λ	-	wavelength

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CHAPTER 1

INTRODUCTION

Delayed luminescence is a method of observing the time-resolved photonic response of biological systems after being exposed to a light stimulus. Previously, this method was used to study cells from microorganisms and plants. As it is different from conventional spectroscopy methods such as transmittance, absorbance, chemiluminescence, bioluminescence, and fluorescence, the mechanism behind delayed luminescence response is not yet fully understood. Despite that, characterizations of delayed luminescence of various samples were researched and presented for various normal and stressed conditions. Recently, delayed luminescence is applied to the photon time-resolved response of human body ranging from organs, tissues, cell and various bodily fluids including surfaces of the skin from various parts of the body. Known also as time-resolved re-emission or time-resolved fluorescence, this method has been shown be able to distinguish between normal and stressed biological systems (Kobayashi and Inaba, 2001; Goi *et al.*, 2007; Scordino *et al.*, 2014). This method is considered non-destructive to the sample under observation as no chemical reagent is used as in common chemical reacting photo analyzers. The delayed luminescence response curve from a wide range of previous studies has shown to be able to distinguish the different conditions of the samples.

1.1 Problem Statement

Recently, saliva has been suggested to be the medium for early disease detection, as an alternative to the current extensive blood sampling, which may cause trauma especially when repeated sampling is required for diagnosis. Diseases screened using saliva includes periodontal disease and assessing caries risk. Due to various emerging technologies, biomarkers from saliva are found for different diseases, such as cancer, auto-immune diseases, viral diseases, bacterial diseases, cardiovascular diseases and HIV (Lee and Wong, 2009). In addition, tongue swab, which is co-related to the saliva in the mouth, may also provide analysis on halitosis or bad breath. Lack of saliva or hyposalivation may increase bacterial activities on the dorsal tongue, which causes bad breath.

Various methods of analysis of saliva and tongue swab for a wide range of diseases are extensively researched. Other than the common optical methods such as the spectrophotometry, delayed luminescence is a one approach in studying biomedical samples. Delayed luminescence of biological samples holds light much longer than the standard bio-fluorescence and it is shown from reviews that it does not originate from the infrared thermal emission alone because it includes the entire wide visible spectrum including ultraviolet and near infrared range.

Even though the delayed luminescence have been studied in organs, tissues, cell, plasma and blood serum, there are hardly any reports on delayed luminescence of saliva and tongue swab and its spectral characterization. The delayed luminescence method has been shown to correlate to various many chemical biomarkers, but the method alone, was not enough to detect or diagnose specific disease but enough for early detection or screening (Scordino *et al.*, 2014). Therefore, this study suggests the delayed luminescence method to be more appropriate for health screening tool. This study also suggests a prototype development of a portable health-screening tool based on the spectral delayed luminescence method. Furthermore, the experimental setup for every organs, tissues,

cells and blood are different and little or close to none is found for measurement of saliva and tongue swab.

Since no record has been found on the research of spectral delayed luminescence of saliva and tongue swab, the spectral delayed luminescence characteristics of saliva and tongue swab are unavailable. Despite various references of spectral delayed luminescence of plant leaves and human blood, the response from saliva and tongue swab is expected to be different due to the content of the saliva. Therefore, the spectral delayed luminescence of saliva and tongue swab needs to be identified and the specific potential application in biomedical analysis pinpointed. In order to achieve this, the spectral delayed luminescence system specifications for this purpose also need to be identified.

The main focus of this study is about the application of delayed luminescence method. Given that the delayed luminescence is not cell-type specific, the delayed luminescence method itself alone cannot be used to diagnose any specific disease. Despite that, delayed luminescence is able to distinguish normal or diseased condition as a general indicator. Therefore, this suggests health screening to be the most appropriate application. However, health screening requires that the procedures to be taken are comfortable, low-cost and non-invasive. The most suitable candidate is oral swab. The spectral characteristics of the delayed luminescence of oral swabs may have patterns for different oral conditions. Thus the conditions most applicable to the delayed luminescence method for health screening using oral swab need to be identified.

1.2 Objectives

In the construction of spectral delayed luminescence, among the essential components required are the photomultiplier tube (PMT), photon counter module, data acquisition system, the optical filter wheel, the dark chamber, and the light stimulator. In order to obtain the optimal measurement of photon count with reduced ambient noise, the PMT needs to be cooled below freezing point. However, this requires complex setup using apparatus to create vacuum condition and specific material for the chamber windows. Previous research used UV light source from nitrogen laser setup for light stimulation. In addition, an optical fiber guide setup is essential to provide the pathway for the UV light to be the channel through the sample in measurement. Exclusion of the PMT cooling module and replace of the complex UV laser with a UV LED may reduce complexity, the size, and power of the spectral delayed luminescence system.

Therefore, in order to address the problems, the main objectives of this thesis are as follows:-

1. To develop the prototype of spectral delayed luminescence system for saliva and tongue swab.
2. To measure and test the significance of omitting PMT cooling in the measurement of delayed luminescence and selecting UV LED against white LED for sample stimulation
3. To identify the potential application of spectral delayed luminescence of saliva and tongue swab by model fitting and performing classification tests using the PLS-DA method

At the end of this research, a prototype platform for the spectral delayed luminescence characterization of tongue swab and saliva samples is made available for future research works. Preliminary spectral delayed luminescence characteristics

of saliva and tongue swabs obtained are to be used as a reference for further in-depth study of the delayed luminescence research for these oral samples.

1.3 Research Scopes and Limitations

In order to set up the boundaries for the above objectives, the research scopes and limitations are stated as follows:-

1. The sample chamber is developed to accept and measure the spectral delayed luminescence of oral swabs obtained on standard cotton swabs.
2. This study is focused on indicating the potential application of the spectral delayed luminescence method for saliva and tongue swab and not for a population clinical study. Saliva and tongue swab are obtained from volunteers with no chronic diseases, but with different conditions of oral health.
3. LEDs (light emitting diodes) are employed of light stimulus instead of high power sources of lights. The focus of the study is directed onto optical spectral response as features for model fitting and classification test and no cross-correlation of biochemical markers is involved

1.4 General Methodology

The general methodology within the research scopes is stated as follows:-

1. The delayed luminescence system shall be developed in two prototypes. The first prototype is a single all-pass delayed luminescence system where the photon counts include all photons with the wavelength ranging from 400 nm to 715 nm. The second prototype shall be able to break up the range into 17 wavelengths; hence, the name spectral delayed luminescence system.
2. The first prototype was tested for the effect of the PMT cooling for a range of stimulation time. Subsequently, it is followed by several tests to determine the selection of the type of LED for sample stimulation.
3. The position of the optical filter disk in the hardware setup of developed second prototype shall be calibrated. The optical filter disk is also calibrated against colored LEDs with their known peak wavelengths. The stimulation time of the saliva and tongue swab is then determined through iteration. And the optimal time shall be employed throughout the subsequent experiments.
4. Saliva and tongue swabs were collected from the volunteers with a range of oral conditions. Then, the models of these conditions were calibrated using the PLSDA classification toolbox in MATLAB. These models were then tested for their classification performance against repeated measurements of the same saliva and oral swabs. The best performance shall indicate the potential application of spectral delayed luminescence using the oral swab for health screening.

1.5 Significant Contributions

Throughout this study, the main objectives have been achieved with significant contributions as follows:-

1. Spectral delayed luminescence system has been developed to specifically measure the oral samples on cotton swabs. This approach eliminates maintenance and storage of saliva and introduces simplicity in the sample preparation process without requiring common sample processes such as cell or tissue culture, refrigeration, and centrifuge. In addition, this system also enables measurement of low-density oral swabs.
2. PMT cooling aids in reducing the ambient photon count unrelated to the test sample but results show that with PMT cooling, the photon counts were not persistent for repeated measurements. PMT operated at room temperature yields in photon counts with lower deviations, which subsequently results in reproducible delayed luminescence measurements. Therefore, the complexity of building the delayed luminescence system is reduced and at the same time, the functionality of this system is maintained.
3. Novel application of spectral delayed luminescence of oral samples on cotton swabs is introduced. From the conducted experiments, results showed that this method may be useful for screening of mouth sores such as mouth ulcer or other conditions related to mouth sore. The high performance of this method is dependent on both spectral delayed luminescence of saliva and tongue swab.

1.6 Thesis Organization

This thesis is organized into six chapters. The current chapter describes the introduction of the research, the research background, and the problems as well as the objectives and the scopes of this research.

Chapter 2 reviews the delayed luminescence method, photon counting system designs for various samples and conditions, and the discussions on the mechanisms of the delayed photon re-emission.

In Chapter 3, the methodology of the research is discussed in detail. This includes the design of the photon counting acquisition unit, the optical filters disc, and light stimulus control systems, and the enhancement made to the second prototype.

Chapter 4 describes the calibration of the system and the experimental tests done to evaluate the feasibility of the system. Experimental tests are carried out in order to determine the effects of operating temperature conditions, to reference the delayed luminescence of saliva to that of distilled water comparing white and ultraviolet LED for stimulus, obtain the spectral delayed luminescence response of the saliva and tongue swabs, and perform model fitting and classifications tests for a number of classes of conditions.

Chapter 5 presents the results of the experiments carried out. The analyses are also brought into discussion in this chapter. The results analysis will be a reference for future experimental setup for more in-depth study.

Finally, Chapter 6 concludes the research works done for this study and put forward suggestions for future works and recommendations in the development of spectral delayed luminescence system specialized for saliva and tongue swab.

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