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Biological Assessments by Innovative Use of Multi-Wavelength Photoplethysmographic Signals Time Differences

Nader Vahdani-Manaf
Department of Electrical and Electronics Engineering, Faculty of Engineering, Karadeniz, Technical University, 61080, Trabzon, Turkey

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Corresponding Author:
Nader Vahdani-Manaf
Department of Electrical and Electronics Engineering,
Faculty of Engineering, Karadeniz,
Technical University, 61080,
Trabzon
Tel : +90 5369796043
Fax : +90 462 325 7405

ABSTRACT

A new biological assessment technique was presented by novel use of photoplethysmography (PPG) signals. Most previous PPG signal analysis techniques were based on morphological and frequency characteristics of PPG. In the present study, time differences (TDs) between separate pairs of PPG signal (with different wavelengths) were used as the novel aspect of PPG technology. A computer connectable multi-channel PPG device and a MATLAB® based program were used to record transmission mode PPG signals with wavelengths of 940 nm (IR), 660 nm (red), 520 nm (green) and 450 nm (blue). The TDs between three different pairs of the signals were obtained during a breath holding experiment (as biology challenge test) to investigate and compare their Time Difference (TD) variations within the tests. The experiments were performed with the participation of 25 healthy subjects. Results showed that TDs of PPG signal pairs had significant variations (p<0.05) in reply to biological challenges. It was concluded that TD variations can be indicative of very important biological information. A good attribute of PPG TD method was its independence from signal amplitude and avoiding from corresponding disadvantage.

Key words: Biological assessments, multi-wavelength PPG, TD variations

INTRODUCTION

Photoplethysmography (PPG) is a very important biological/physiological evaluation technique. It is an opto electrical method to measure blood volume variations in the biological tissues (caused by microvascular expansion and contractions) nearby the skin surface (Krishnan *et al*., 2010). The PPG signal comes from the interplay of light with the blood vessels. Some optical processes such as scattering, absorption, reflection, transmission and fluorescence are involved in the PPG (Allen, 2007; Anderson and Parrish, 1981). Principal human biological information is included in the PPG and so, analyzing the PPG signal is a major procedure to assessment the status of human physiology (Shi *et al*., 2010).

Until recent decades, PPG signal was used for the evaluation of limited biological parameters. However, in more recent years, novel methods were presented to use the PPG as an important biological and clinical assessment technique (Bagha and Shaw, 2011; Asada *et al*., 2003). In the previous studies, general goals of PPG applications were obtaining of biological information and use of PPG as an alternative to other bio-signals such as electrocardiography and electro-plethysmography. Blood oxygen saturation (Kyriacou *et al*., 2002), pulse number (Selvaraj *et al*., 2008), respiratory rate (Johansson, 2003) and blood pressure (Binns *et al*., 1995) were the major PPG parameters studied in research. Furthermore, PPG could contain some other significant cardiovascular features, such as arterial stiffness (Pilt *et al*., 2013), cardiac output and systemic vascular resistance (Lee *et al*., 2013). Until to now many properties of PPG were analyzed, but attributes and physiological characteristics of PPG signal not fully discovered yet (Li *et al*., 2014).

Overall, important specifications of PPG signal that previously were used to biological measurements can be classified as morphological characteristics, time and frequency domain characteristics and amplitudes ratios. Various methods have been proposed to use of the mentioned PPG characteristics, the most common of which are extraction of heart rate and heart rate variability (Scully *et al*., 2012), pulse oximetry (Taylor and Whitwam, 1986) and using the morphology of PPG signal (Linder *et al*., 2006; Yousef *et al*., 2012).

In the research, there are a few works based on concurrent different PPG signals for biological assessment applications. This type of PPG usages was usually employed for pulse oximetry using amplitude ratios of two PPG waveforms with distinct wavelengths (Phillips *et al*., 2009), analyzing vascular pressure variations at different depths of human skin (Asare *et al*., 2012a) or analysis of skins blood circulation specifications (Asare *et al*., 2012b).

Despite the fact that two or more wavelength PPG signals have been used in some of previous studies, none of those works have concentrated the time difference changes (phase shifts) of distinct simultaneous PPG signals relative to each other in terms of individual's biological conditions. In this paper, as the novel aspect of PPG, variations of beat to beat TDs between the concurrent separate PPG signals with different wavelengths were analyzed during biology challenge tests. According to results, presented method can have a potential for physiological and clinical applications such as apnea diagnosis, breath studies, respiration disorders or measuring of blood parameters.

MATERIALS AND METHODS

Data collection and experimental protocol: Data collection was carried out by using a computer connectable multi-wavelength transmission mode PPG signal recorder device with a ring-shaped sensor. Three separate pairs of simultaneous PPG signals with the wavelength pairs of 660-94 nm (red-IR), 520-940 nm (green-IR) and 450-940 nm (blue-IR) were selectable by this PPG recorder. In addition to the device, a host PC installed MATLAB® program was developed for signal recording and processing. Time duration of signal recordings, wavelength pairs and also sampling frequency could be changed via this program.

Experimental protocol was needed to assess the multi-wavelength PPG time difference variations with respect to biological situations. Breath holding is a major biological challenge in which blood dissolved gases are changing seriously (Rassovsky *et al*., 2006). In breath holding, the blood dissolved of oxygen declines under its ordinary rate and that of carbon dioxide rises above its usual amount (Parkes, 2006). Extent of these changes depends on breath holding duration and amount of stored air in the lungs (Klocke and Rahn, 1959). Hence, breath holding experiment was performed for instance of biological changes occurring conditions. The experiment was contained steps of 1 min normal breathing and holding the breath as much as possible. Timing of steps of the experiments was performed by a digital stopwatch.

Experimental setup to collect PPG signals containing recording device, a laptop and related MATLAB® program is revealed in Fig. 1. Experimental recordings were obtained with the participation of 25 healthy volunteers [age = 26.4±12.3 years (Mean±SD), range 20-39 years, male/female = 9/6]. Before beginning to record signals, subjects were seated and relaxed on a chair, ring-shaped sensor was placed in a motionless form on their right hand forefinger and then, recording was began and the subjects were asked to execute the mentioned steps of the current experiment. All of the signals were recorded at the same laboratory with the room temperature approximately 24°C. Sampling frequency of signal recording was 1 KHz.

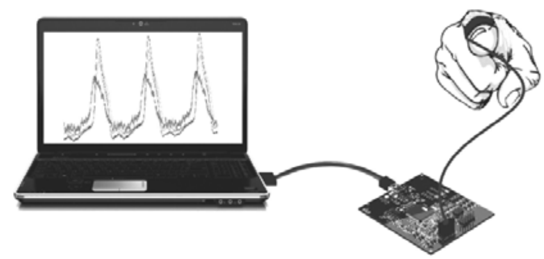

Fig. 1: Experimental setup used for data collection

Filtering, peak detection and time difference calculation: The process of signal filtration depends on frequency specifications of the signal. Regarding this fact that PPG signals are an important effect of arterial system (O'Rourke, and Taylor, 1966), the basic frequency of PPG has been demonstrated to be about 1 Hz (Lee *et al*., 2007; Allen and Murray, 2004). Attention to the frequency characteristics of PPG signals, it could be accepted that a band pass filter in the range of 0.05-5 Hz was proper to PPG signal filtration. Therefore, in this paper, noise clearing process from the experimental recordings was carried out by a band pass filter in the mentioned frequency range.

Finding of peak points is a major task in various signal analyzing processes including the time difference extraction involved in this study. The PPG signal has a relatively simple shape and it seems that isn't hard to peak detection. Nevertheless, major baseline wander and respiration effect may be superimposed on PPG signal. This signal also can be quickly affected by frequently happened physiological oscillations or motions. The conventional methods for peak detection are difficult to exert for quick variations of PPG waveform in various heart rates and they also may have time lag (Shin *et al*., 2009; Lao, 2013) which can interfere with the time difference computing operation involved in this study and led to inaccurate values. Thus, for PPG signal conditioning and peak calculation, adaptive threshold peak detection algorithm (Shin *et al*., 2009) was employed.

Time Differences (TDs) between each pair of PPG signals can be calculated after the peak detection process using; (1) For example, the TD between red and infrared PPG signals was calculated by subtracting the detected peak point time of these PPG pulses in each beat of the heart and (2) TD vectors were formed from the obtained time difference values. The

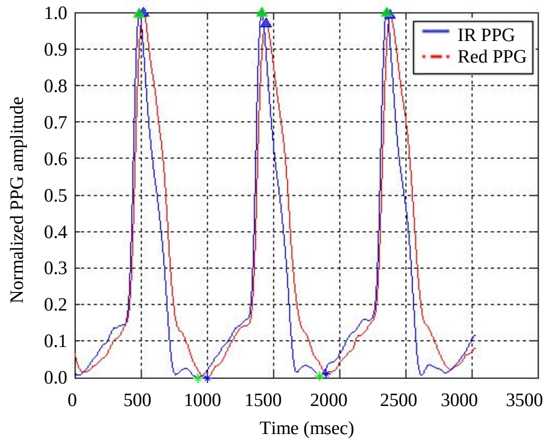

Fig. 2: Peak points of PPG pulses and their time difference

mentioned vectors were obtained for each pair of PPG signals (red-IR, green-IR, blue-IR) separately.

$$t_i = Peak_2 - Peak_1 \quad (1)$$

$$TDs = (t_1, t_2, \ldots, t_i) \quad (2)$$

In (1), t_i, $Peak_1$ and $Peak_2$ denote the time difference between PPG pulses in the i-th beat of heart, peak points of selected pair of PPG pulses, respectively and i is the heartbeat number index. Red-IR PPG pulses pair and their peak points can be seen in Fig. 2 for instance in Eq. 1 and 2:

Statistical analysis: Time domain parameters as mean and standard deviation of measured TDs were calculated for each step of the experimental recordings. The paired t-test is the most frequently used procedure to assess the differences in means between two groups. Differences between breathing spontaneously and breath holding were investigated by independent paired t-test on the calculated parameters. A value of $p<0.05$ was considered statistically significant. Statistical analysis was carried out using of Statistics and Machine Learning Toolbox™ of MATLAB®.

RESULTS AND DISCUSSION

Table 1 represents mean, Standard Deviation (SD) and Standard Error of the Mean (SEM) of TDs for the PPG pairs of red-IR, green-IR and blue-IR separately. As seen in this table, in normal breathing phase (breathing spontaneously), the mean of TDs between the mentioned pairs of different PPGs were about 3.22, 8.12 and 11.84 msec, respectively. In the breath holding, the mean of TDs were reduced. Besides, standard deviations of the TDs were increased in the breath holding relative to the breathing spontaneously.

The results of the paired t-test for each of PPG pairs are shown in Table 2. As seen in this table, p-value for TDs of red-IR PPG pair was 7.1473×10^{-9}. By conventional criteria, this difference is considered to be extremely statistically significant. For green-IR PPG pair the p-value was 0.0015 that was considered to be very statistically significant. In the case of blue-IR PPG, the p-value calculated as 0.0241that this value was considered to be statistically significant. The barplot with error bars obtained from the mean and SEM of TDs related to the each PPG pairs is illustrated in Fig. 3. This figure also indicate that TDs were significantly different for normal conditions (breathing spontaneously) and biological challenging conditions (breath holding).These results confirm that although all three PPG pair had significant biological related TD variations, but the greatest and the most significant TD variations were related to the red-IR PPG pair and TDs of blue-IR pair had the lowest response to biological variations.

Table 1: Mean SD and SEM of TDs

	Breathing spontaneously			Breath holding		
PPG Pair	Mean	SD	SEM	Mean	SD	SEM
Red-IR	3.22	0.89	0.1625	-3.88	3.54	0.6463
Green-IR	8.12	3.63	0.8117	3.18	5.33	1.1918
Blue-IR	11.84	5.95	1.502	4.922	11.69	3.8352

SD: Standard deviation, SEM: Standard error of mean, TDs: Time differences

Table 2: p-value of the paired t-test for PPG pairs

Biological condition pair	Breathing spontaneously-Breath holding
Red-IR	7.1473×10^{-9}
Green-IR	0.0015
Blue-IR	0.0241

Multi-wavelength PPG was considered with different methods by other researchers. Nonetheless, TD is new issue in multi-wavelength PPG technology. The presented method is compared with that of other multi-wavelength PPG studies in terms of the considering the TDs and their variations with respect to biological changes. Table 3 presents the comparison of the proposed to other methods. Some studies such as Phillips *et al*. (2009), Bagha and Shaw (2011) and Kyriacou *et al*. (2002) used the multi-wavelength PPG without considering TDs. The used parameter in these studies was the amplitude ratio of PPG signals.

Multi-wavelength PPG was applied also by Spigulis *et al.* (2007) for skin micro circulation assessment. In this study,

Table 3: Comparison of the proposed to other methods

Used parameter	Statistical significance of TD variations in terms of biological conditions	References
Amplitude ratio	Not studied	Kyriacou *et al*. (2002)
Amplitude ratio	Not studied	Phillips *et al*. (2009)
Amplitude ratio	Not studied	Bagha and Shaw (2011)
Short-term TDs	Not studied	Asare *et al*. (2012a)
Short-term TDs	Statistically significant (p<0.05)	Asare *et al*. (2012b)
Long-term TDs		Presented method

TD: Time difference

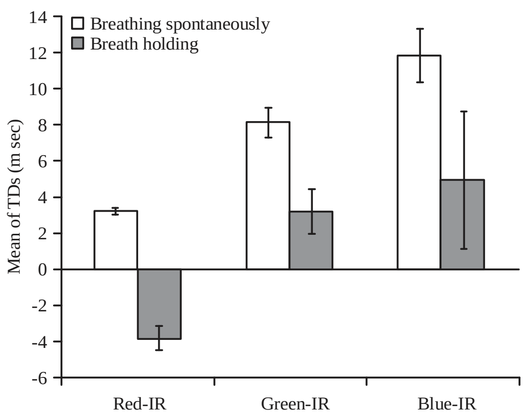

Fig. 3: Mean of TDs for both breathing spontaneously (blue bars) and breath holding (yellow bars). Data are expressed as Mean±95% confidence intervals

different PPG signals have been simultaneously recorded in separate wavelengths. Results of this research showed that signal baseline responses at separate wavelengths was different. They concluded that a depth variety of the skin blood pulsation dynamics was caused to distinct baseline values.

Gailite *et al*. (2008) employed multi-wavelength PPG based on fiber-coupled laser irradiation and time-resolved spectrometric detection. They confirm that PPG pulses that were recorded simultaneously at distinct wavelengths were different in waveform. The outcomes of these two researches can support our results in the case of TDs with the exception that they focus on baseline and pulse shape differences.

The existence of the time difference between peak points of simultaneous different PPG signals obtained from a small hole of the skin is corresponded with the Asare and Spigulis (2013) study, Asare *et al*. (2012a, b) in which multi-wavelength PPG signals were analyzed with respect to blood microcirculation characteristics at different vascular depths. These studies measured short-term TDs between different simultaneous PPG signals manually with no TD extraction algorithm and no respect to the biological conditions.

In addition to the above mentioned studies, Kuzmina *et al*. (2007) assembled and tested a fiber-optic spectrometry set-up for applications in skin diffuse laser fluorescence spectrometry and multi-wavelength photoplethysmography studies. Their results also showed differences in spectra of healthy and pathologic skin that can also support the outcomes of our research.

Natural fluorescence of the blood has been introduced years ago (Fuerst and Jannach, 1965). Fluorescence feature of blood is due to the existence of some elements such as hemoglobin that they have an intrinsic fluorescence property (Parul *et al*., 2000). Gao *et al*. (2004) measured the fluorescence spectra of the whole blood and the hemoglobin. Outcomes of this research revealed that fluorescence spectrum was affected by the wavelength of emitting light and amount of the blood dissolved fluorophores. Considering the results of this research, variations of TDs in distinct biological situations and difference between TDs obtained from separate wavelength pairs (involved in the present study) may be interpreted by the blood fluorescence phenomenon.

The paper uniquely presented a new strategy for the analysis of multi-wavelength Photoplethysmographic signals in terms of biological conditions. In summary, it can be said, with respect to the results it was demonstrated that as long as physiological situation was stable, the TDs had no large variations. However, when the biological state was changed, the mean and standard deviations of TD values were significantly changed. This means that the obtained TD series had a pattern related to biological conditions that can be indicative of human physiology. It seems that TDs and their biology-related pattern can be used for important clinical applications.

CONCLUSION

A novel method was presented for biological analysis by innovative use of the multi-wavelength PPG signals. It could be concluded that, TDs may be indicative of many important physiological and biological information and usage of them is quite feasible for biological measurements and/or diagnostic applications. Based on the results, all of the three PPG pair of red-IR, green-IR and blue IR had significant biological related TD variations, but the red-IR pair was the most relevant case for biological applications. An important characteristic of the introduced method was its independence from the light intensity and abstaining from several draw backs such as sensitivity to light source or sensor drift, optical path variations and attenuation of light source.

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