Quantifying low-frequency fluctuations in the laser Doppler flow signal from human skin

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ABSTRACT

Low-frequency fluctuations in the laser Doppler flow signal (LDFS) from the skin are related to microvascular mechanisms of flow control. Wavelet spectral analysis has been used to correlate fluctuations in the LDFS with the endothelial, neurogenic and myogenic mechanisms of control in the frequency intervals 0.005-0.02 Hz, 0.02-0.06 Hz and 0.06-0.16 Hz, respectively. Generally the signal power, in each frequency interval, derived from the respective wavelet coefficients, is used as a measure of the activity of the related mechanism of microvascular control. However, the time-domain characteristics of the fluctuations in the LDFS in each frequency interval are poorly known. As a consequence, there is a lack of objective criteria to properly measure, in each frequency interval, the related hemodynamic parameters. Here a time-domain method is proposed to analyze and quantify fluctuations in the LDFS in each frequency band. Baseline (32 degrees Celsius) and thermally stimulated (42 degrees Celsius) LDFS of forearms from 15 healthy volunteers were collected and analyzed. The data obtained indicate that inappropriate time windows, frequently used for measurements, increase the variability of the measured signal power, diminishing the capability of the method when assessing microvascular dynamics and dysfunctions. To overcome this limitation, an objective method to measure the LDFS power in each frequency band is proposed.

Keywords: laser Doppler, vasomotility, skin blood flow

1. INTRODUCTION

The laser Doppler flowmetry is a noninvasive method frequently used for studying the skin blood flow. The laser Doppler flow signal from the skin generally contains constant and oscillatory components. Cyclical flow variations have different periods (and frequencies), according to their origins. Spectral analysis has been used to identify six frequency bands, around 0.008 Hz, 0.01 Hz, 0.04 Hz, 0.1 Hz, 0.3 Hz and 1 Hz, whose origins are known: i) endothelial, ii) endothelial NO-dependent, iii) neurogenic, iv) myogenic, v) respiratory and, vi) cardiac, respectively [1].

Recent studies have shown that the amplitude of the fluctuations in the frequency range around 0.01 Hz is a parameter that discriminates microvascular endothelial dysfunction even when a measurable change of the microvascular flow to a vasodilator stimulus is not detected, suggesting that flow oscillation could detect early endothelial changes [2].

The identification of spectral bands was initially performed using the short-time Fourier Transform. Currently the most common method of analysis is the wavelet transform. The method is also used to quantify the oscillations: the wavelet coefficients are calculated and time-integrated (e.g., during 300 s). The time-integrated wavelet coefficients are frequency-integrated (e.g., from 0.02 to 0.05 Hz) and the resulting quantity (P) has been used as an indicator of the activity for the related selected spectral range (in the example above, related to the neurogenic activity). The quantity (P) is proportional to the power of the oscillating flow in the considered spectral range. The variation of (P) to stimuli that result in vasodilation has been studied in healthy subjects and in various pathologies. However, the quantity (P) is the mean power of the flow fluctuation in a selected frequency band and this method does not allow the study of the temporal response of microvascular flow to a stimulus. Recently the wavelet analysis in short time-intervals [3] and adaptive wavelet transform [4] were proposed for analyzing the flow oscillations in the time domain. The analysis of temporal fluctuations of flow in spectral bands, preserving the phase and amplitude of the signal in each spectral band, is beyond of the scope of these works. In addition, the time interval to which the average power (P) is calculated has been arbitrary selected. The aim of this paper is to present an alternative method for analysis and quantification of flow oscillations in the time domain. The method was evaluated in healthy volunteers.

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2. METHODS

2.1 Flow signal processing

In order to separate the flow signal into six spectral bands, according to their origins, second-order Butterworth filters were implemented using the MatLab R2009b. Six band-pass filters according to Table (1) were implemented. A second-order Butterworth low-pass filter with a cutoff frequency equal to 0.005 Hz was also implemented and the resulting signal contains the DC portion and very slow fluctuations with frequencies below 0.005 Hz.

Table 1: Frequency bands of the filters used according to their origins.

Band	Frequency interval (Hz)	Origin
B0	0 - 0.005	
B1	0.005 – 0.0095	Endothelial
B2	0.0095 – 0.02	Endothelial, NO-dependent
B3	0.02 – 0.06	Neurogenic
B4	0.06 – 0.16	Myogenic
B5	0.16 – 0.4	Respiratory
B6	0.4 – 1.6	Cardiac

Usually the flow time series are collected and stored in a computer for further analysis. In this condition, zero-phase digital filters can be implemented by processing the time series in the forward and backward directions [5]. The resulting filter order is the doubled. Thus the implemented second-order filters are in fact fourth-order filters. We used two fourth-order filters in sequence, so that the resulting filter order is eight.

As can be seen in Table (1), the cutoff frequencies of the filters are low, when compared to the sampling frequency of the signal (100 Hz to minimize quantization noise), then down sampling was performed using a factor 25, resulting in sampling frequency equal to 4 Hz. In addition to avoid filter instabilities due to its low cutoff frequencies, instead of implementing filters of order eight, two sequential fourth-order filters were implemented (as above described).

While the implementation of the wavelet transform requires the removal of the DC portion of the Doppler signal [6], filtering in the time domain requires the removal of signal transients. As it will be shown later, oscillations around 1 Hz have high amplitudes, and cause disturbances in the filters with lower cutoff frequencies. Thus, the Doppler signal was pre-filtered through a Butterworth fourth-order low-pass filter, with cutoff frequency equal to 0.02 Hz, before being filtered through the two filters with lower cutoff frequencies (low-pass, cutoff frequency of 0.005 Hz and band-pass, frequency range from 0.005 to 0.0095 Hz). This way stable filters that preserve the phase and the amplitude of the flow signal were obtained.

2.2 Measurements

The flow measurements were performed using a laser Doppler flowmeter (MoorLab, Moor Instruments Ltd., UK), equipped with a laser emitting at 780 nm (infrared). The laser Doppler flowmeter cutoff frequency used was set at 15 kHz. The probe used was the model MP12-V2 (Moor Instruments Ltd., UK). The probe was inserted in a plastic disc and fixed in the investigated area using double-sided adhesive. The probe holder (plastic disc) has a small electrical resistance, used to heat a small region where the flow is to be measured. Temperature control was accomplished using a local heating system (Moor Instruments Skin Heating Unit SH02, Moor Instruments Ltd., UK). Flow values are shown as arbitrary perfusion units (AU), calibrated against a standard flow according to the instructions of the flowmeter manufacturer.

In order to evaluate the method of analysis, flow registers were performed in the volar forearms of 15 healthy volunteers of both sexes, aged from 27 to 36 years, seven females (mean age 32.3 ± 2.9 years) and eight males (mean age 31.9 ± 3.1 years). The exclusion criteria were: diabetes, vascular disease, hypertension and use of vasoactive medicines.

Informed consent was obtained from each subject prior to the measurements. During all tests room temperature was maintained at 23 ± 1 ^{0}C.

The flow signals were digitized (16 bit, 100 samples/s) using a multifunction card NI USB-6255 (National Instruments, USA) and stored in a computer.

2.3 Stimulus

The stimulus method used was the local thermal stress, where an electrical resistance heated a small region (approximately 2 cm^2). The heating rate used was approximately 1 ^{0}C/min., resulting in no discomfort to the volunteers.

An example of flow record is shown in Figure (1), following the protocol of heat stress used: the basal flow is measured during approximately five minutes when the skin temperature was 32±1 ^{0}C (room temperature controlled at 23 ^{0}C). The local heating (32 ^{0}C to 42 ^{0}C) causes a biphasic flow response as shown in Figure (1). It has been shown that the origin of the first peak is neurogenic and the plateau is dependent on the release of NO from the vascular endothelium [1]. Thus, the thermal stress is a promising method to assess endothelial and neurogenic responses and this is the reason for choosing this method of stimulation. Records of flow without heat stimulation were also performed during another session lasting approximately 30 minutes, to characterize the basal flow (without stimulation).

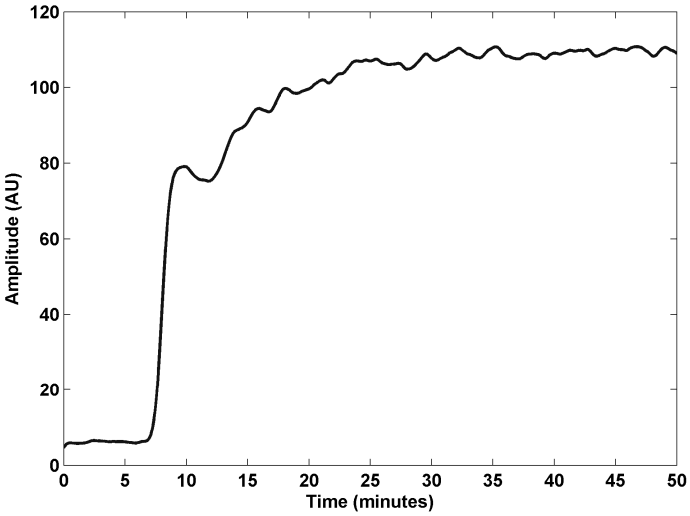

Figure 1: Skin blood flow record following the thermal stress protocol used. The origin of the first peak is neurogenic and the plateau is dependent on the release of NO from the vascular endothelium.

3. RESULTS AND DISCUSSION

In Figure (2) are shown a flow record, taken during approximately 50 minutes, divided into seven frequency bands (B0-B6) according to Table 1. As can be seen, except in the frequency band B6 (cardiac), the amplitudes vary slowly and widely during the registration. This fact is evident in Figure (3), where the envelope of the absolute value of the signal in the B2 band (endothelial NO-dependent) is drawn.

Since the amplitude of each signal varies slowly and largely, its RMS value also varies depending on the time interval and location where the average is taken.

In Figure (4) in shown the deviation of the RMS amplitude (m1) of the signal in the Figure (3), calculated using a time-average interval equal to one minute taken in the time interval from 15 to 50 minutes (after heating), when compared to the RMS amplitude of the same signal (m50) when computed over the entire time interval from 15 to 50 minutes. That is, the percentage deviation shown in Figure (4) is (m1/m50) x 100 and the time interval in which m1 is

calculated continuously varied between 15 minutes and 50 minutes. As can be seen, deviations higher than 100% were observed. Thus the time domain analysis revels very slow and large amplitude changes, suggesting that longer time-average intervals than those normally used should be considered ([3] use one minute and [4] use five minutes).

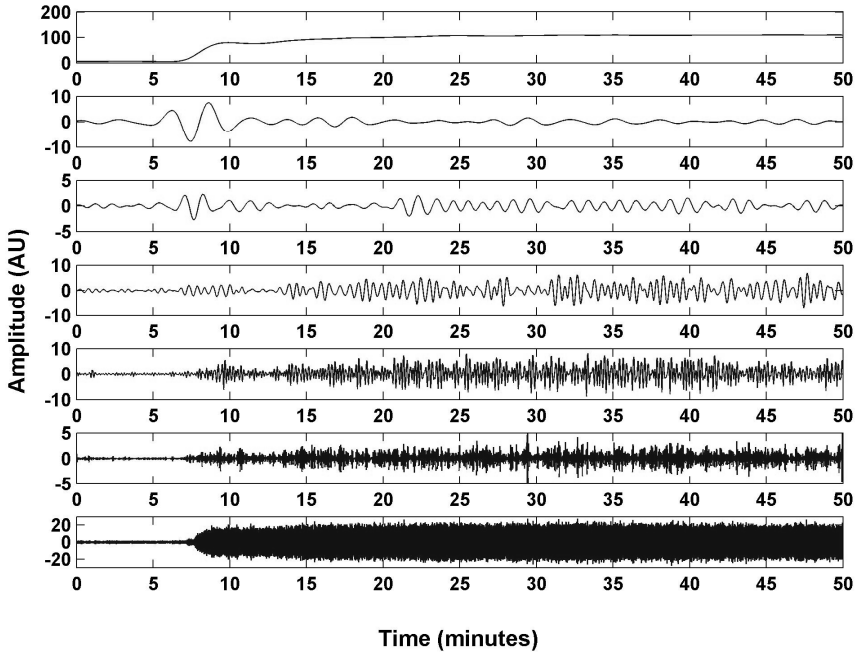

Figure 2: Skin blood flow signal during a stress heating protocol, divided into seven frequency bands: B0-B6 from top to bottom (see Table 1).

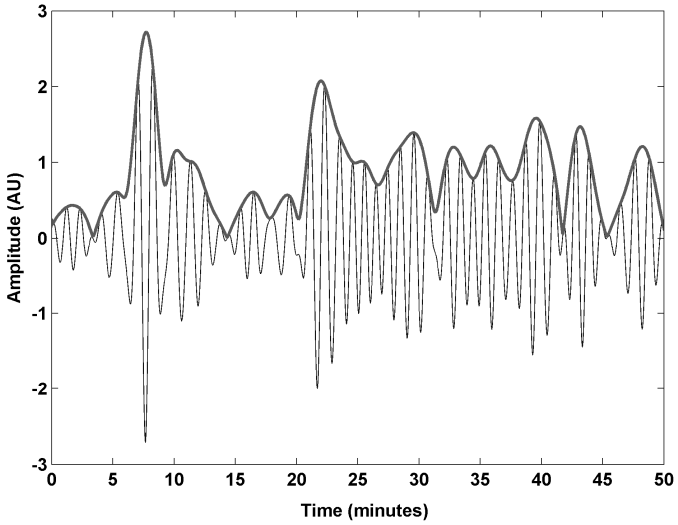

Figure 3: Skin blood flow oscillation in the frequency range from 0.0095 to 0.02 Hz, The envelope emphasizes the slow and large amplitude fluctuation.

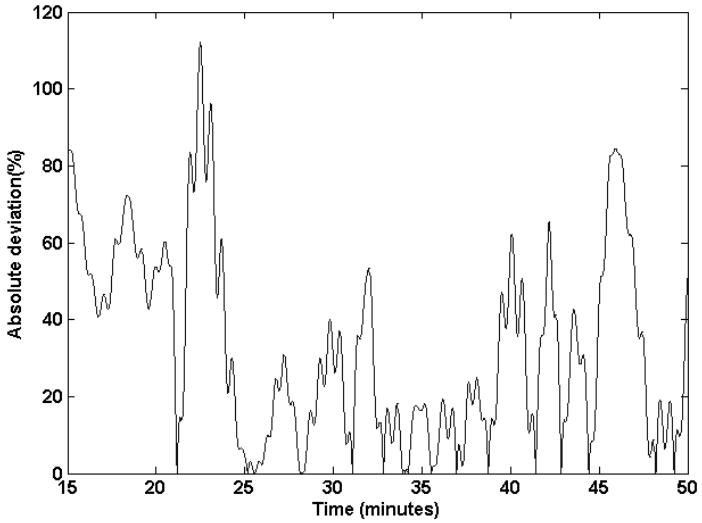

Figure 4: Percentage of deviation (m1/m50) x 100 where m1 is the RMS amplitude of a flow signal in the B2 band computed using a time-average interval of one minute and m50 is the RMS amplitude of the same signal using a time-average interval of 35 minutes. The window location (time-average interval of one minute) varies from 15 minutes to 50 minutes.

In Table (2) is shown the mean values of the RMS amplitudes of the measured flows divided in seven bands (B0-B6), calculated using time-average intervals of 20 minutes. Records were made with the local temperature equal to 32 ^{0}C (basal) and equal to 42 ^{0}C (heat stress).

Table 2: Mean values of the RMS amplitudes of flow values recorded from fifteen health volunteers divided into seven frequency bands (B0 – B6). SD means Standard Deviation and CV Coefficient of Variation (SD/mean).

	Flow (local temperature = 32 ^{0}C)			Flow (local temperature = 42 ^{0}C)		
	Amplitude (AU)	SD (AU)	CV	Amplitude (AU)	SD (AU)	CV
B0	5.83	1.64	0.28	110.49	40.62	0.37
B1	0.23	0.07	0.32	1.07	0.39	0.36
B2	0.22	0.06	0.27	1.26	0.57	0.45
B3	0.42	0.16	0.38	1.75	0.66	0.38
B4	0.44	0.17	0.39	2.07	0.79	0.38
B5	0.26	0.09	0.36	1.8	0.7	0.39
B6	0.81	0.36	0.45	12.35	4.65	0.38

The presented RMS values are not normalized. It is a common procedure normalizing the amplitudes (or power) by the blood pressure or other parameters, aiming to reduce variability, which can be evaluated observing the values of the

coefficient of variation (Standard Deviation/mean) expressed in Tab (2). This is a subject of interest in another current work of our group. To our knowledge, it is the first time that mean values of the RMS amplitudes of the Doppler-signal oscillations from forearms are presented. This way it was not possible to compare the obtained values with other results. Since the filters used preserve the amplitudes of the filtered signals, the method is also useful to properly specify the resolution needed for the analog-to-digital conversion. The presented method expands also the possibilities of analysis of the fluctuations in flow signals. The new possibilities of study include the analysis of the temporal correlation of fluctuations in different regions of the same individual, because the filters used preserve the phases of the signals.

4. CONCLUSION

This paper presents a method intended for temporal analysis of flow signals obtained by the laser Doppler flowmetry. The method preserves the phase and amplitude of the signal, increasing the possibility of studying the microcirculation via laser Doppler flowmetry.

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