Time of correlation of low-frequency fluctuations in the regional laser Doppler flow signal from human skin

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ABSTRACT

The laser Doppler flowmetry allows the non-invasive assessment of the skin perfusion in real-time, being an attractive technique to study the human microcirculation in clinical settings. Low-frequency oscillations in the laser Doppler blood flow signal from the skin have been related to the endothelial, endothelial-metabolic, neurogenic and myogenic mechanisms of microvascular flow control, in the range 0.005-0.0095 Hz, 0.0095-0.021 Hz, 0.021-0.052 Hz and 0.052-0.145 Hz respectively. The mean Amplitude (A) of the periodic fluctuations in the laser Doppler blood flow signal, in each frequency range, derived from the respective wavelet-transformed coefficients, has been used to assess the function and dysfunctions of each mechanism of flow control. Known sources of flow signal variances include spatial and temporal variability, diminishing the discriminatory capability of the technique. Here a new time domain method of analysis is proposed, based on the Time of Correlation (TC) of flow fluctuations between two adjacent sites. Registers of blood flow from two adjacent regions, for skin temperature at 32 ^{0}C (basal) and thermally stimulated (42 ^{0}C) of volar forearms from 20 healthy volunteers were collected and analyzed. The results obtained revealed high time of correlation between two adjacent regions when thermally stimulated, for signals in the endothelial, endothelial-metabolic, neurogenic and myogenic frequency ranges. Experimental data also indicate lower variability for TC when compared to A, when thermally stimulated, suggesting a new promising parameter for assessment of the microvascular flow control.

Keywords: laser Doppler, vasomotility, skin blood flow

1. INTRODUCTION

The laser Doppler flowmetry is a noninvasive technique, frequently used to study the skin blood flow. The amplitude of the laser-Doppler flow signal from the skin at a fixed site is a time-variable quantity, following the circulatory and respiratory cycles. The flow signal exhibit also cyclical fluctuations in frequency ranges 0.005-0.0095 Hz, 0.0095-0.021 Hz, 0.021-0.052 Hz and 0.052-0.145 Hz, related respectively to the endothelial, endothelial-metabolic, neurogenic and myogenic mechanisms of microvascular flow control.[1]

Functions and dysfunctions of each mechanism of flow control have been assessed, by analyzing the amplitude (A) of the time-average and frequency-average wavelet-transformed coefficients of the fluctuating signals, in each frequency range.[2]

The capability of the technique to discriminate an abnormal microvascular function from normal responses is diminished by the signal variability. Know sources of flow signal variability includes spatial heterogeneities of the microvascular plexus[3] and temporal fluctuations of the signal of flow.[4] Temporal fluctuations can be diminished by (adequately) time-averaging the flow signal, filtered in each of the above mentioned spectral ranges [4], or similarly, by time-averaging the wavelet coefficients.[5] In spite of this fact, time-windows for averaging the amplitude, A, have been arbitrary selected.[4, 5]

Recently a time-domain method for characterization of the laser Doppler flow signal fluctuations in the above mentioned spectral ranges was proposed[4], aiming to control errors due to inappropriate time-windowing (for time-averaging). The aim of this paper is to suggest a time-domain method to compute the time of correlation (TC) of the low-frequency components of the skin flow signal from two adjacent regions and to compare TC variabilities with those from the A parameter.

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2. METHODS

A laser Doppler flowmeter (MoorLab, Moor Instruments Ltd., UK) was used for flow measurements. The flowmeter is equipped with a laser emitting at 780 nm (infrared). The cutoff frequency of the Doppler filter was set at 15 kHz. Two probes model MP12-V2 (Moor Instruments Ltd., UK) were use for measurements. The probes ware inserted in plastic discs and fixed in the investigated areas using double-sided adhesive. Each probe holder (plastic disc) has a small electrical resistance, used to heat a small region where the flow is to be measured. The local temperature was controlled 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.

A multifunction card NI USB-6255 (National Instruments, USA) was used to digitize the flow signals (16 bit, 100 samples/s). Digitized signals were stored in a computer for posterior analysis.

Flow registers were performed in two sites (spaced ~ 5 cm apart) of the volar forearms of 20 healthy volunteers aged from 27 to 36 years: ten females (mean age 32.1 ± 2.7 years) and ten males (mean age 31.7 ± 3.1 years). The exclusion criteria were: smoker, diabetes, vascular disease, hypertension and use of vasoactive medicines. The study was approved by the local ethics committee. Informed consent was obtained from each subject prior to the measurements. During all tests room temperature was maintained at $23 \pm 1\ ^0C$.

Flow measurements were performed at 32 ^0C (basal) and heated to 42 ^0C (skin temperatures). The electrical resistances of the probe holders heated small regions (approximately 2 cm^2). The heating rate used was approximately 1 ^0C/min., resulting in no discomfort to the volunteers. Measurements were performed in two sessions (basal and thermally stimulated), each register lasting from 20 to 30 minutes. The thermally stimulated measurements were carried out ten minutes after the start of the thermal stimuli, to allow stabilization of the fluxes (plateau).

The digitized flow signals were processed according to Folgosi-Correa et al.[4] The block diagram of the signal processing used is shown in FIG. 1. Briefly, the sampling frequency was decimated to 4 Hz and the flow signals were filtered by fourth-order Butterworth filters, implemented as zero-phase digital filters using MatLab R2009b. A zero-phase filter preserves the signal phase and amplitude. The band-pass frequencies of the implemented filters are shown in TAB. 1.

Since signals in the bands B1 to B4 have zero mean (because the DC levels were filtered out), the mean of their absolute values were computed.

A normalized measure of dispersion was used as a measure of the variability of the absolute value of the signals: the coefficient of variation, defined as $CV = SD/m$ where SD and m are standard-deviation and mean respectively.

To verify possible linear relationships of the signals from the two measured adjacent regions (forearms of volunteers), in each band (B1 to B4), the analysis of cross-correlation with zero-delay (Pearson product-moment correlation) was used, modified from Corsi-Cabrera et al.[6] Briefly, the method was performed in two steps. First, a movable time-window selects a segment of the two signals in each band (B1 to B4). Secondly, the Pearson product-moment correlation (R) is estimated on each pair of signals from the two investigated regions. As the time-window moves from the beginning to the end of the signal, a time-dependent correlation coefficient is obtained. Movable time-windows, with durations T equal to 200 s, 100 s, 50 s and 17 s, for the signals in the bands B1 to B4 respectively, were used to compute correlations.

Significant correlation was assumed for $R > 0.5$, and the percentage of time that a pair of signals remained correlated during each experiment (lasting 20 to 30 minutes) was computed, here denoted as percentage of time of correlation (PTC). Similarly as above described, CV was used as a normalized measure of dispersion of the PTC.

Comparisons of PTC values were performed using paired t-Student tests, after checking the normality of the samples (Shapiro-Wilk normality test).
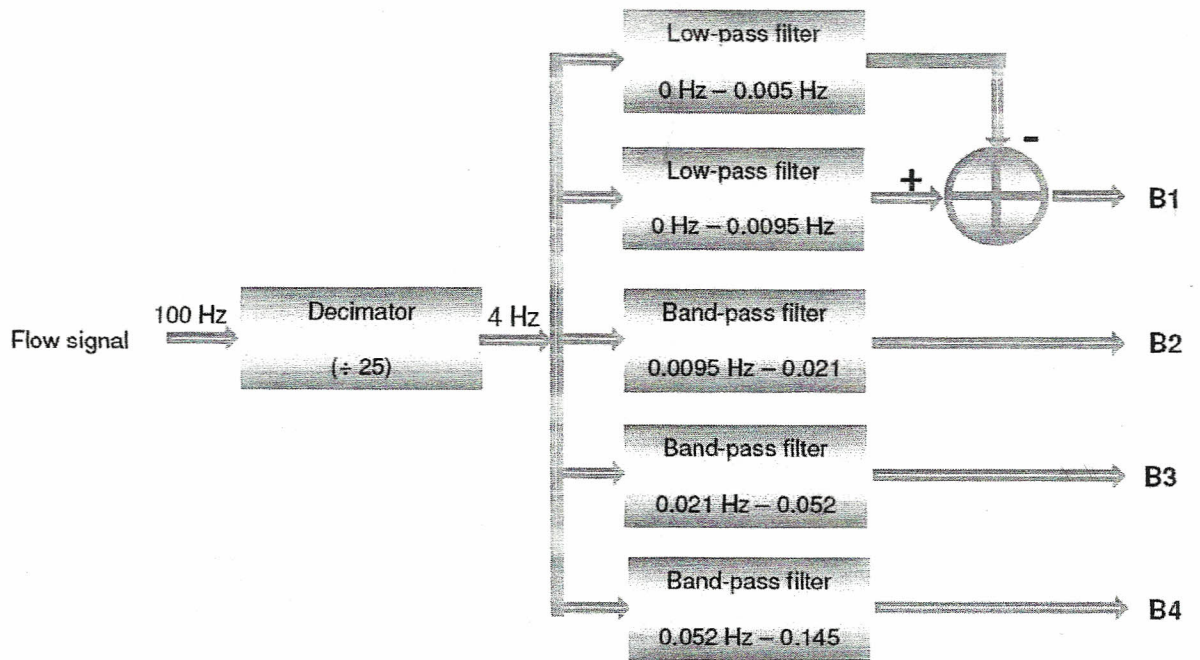
Figure 1: Block diagram of the filters used to separate the flow signal in four spectral bands B1 to B4.

Table 1: Frequency ranges of the band-pass filters used, according to their origins.

Band	Frequency Interval (Hz)	Origin
B1	0.005 – 0.0095	Endothelial
B2	0.0095 – 0.021	Endothelial-metabolic
B3	0.021 – 0.052	Neurogenic
B4	0.052 – 0.145	Myogenic

3. RESULTS AND DISCUSSION

Pairs of blood-flow signals, from two adjacent regions of a volunteer, filtered in four spectral bands (B1 to B4), during a thermal stress, are shown in FIG. 2. The visual inspection reveals intermittent synchronism between all pairs of signals.

For each pair of signals, the observed time-interval of continuous synchronism was variable among individuals and spectral bands, ranging from tens to hundreds of seconds, although their amplitudes differed. The same pattern (intermittent synchronism) was observed in all registers studied, although thermal stimulation resulted in longer time intervals of synchronism.
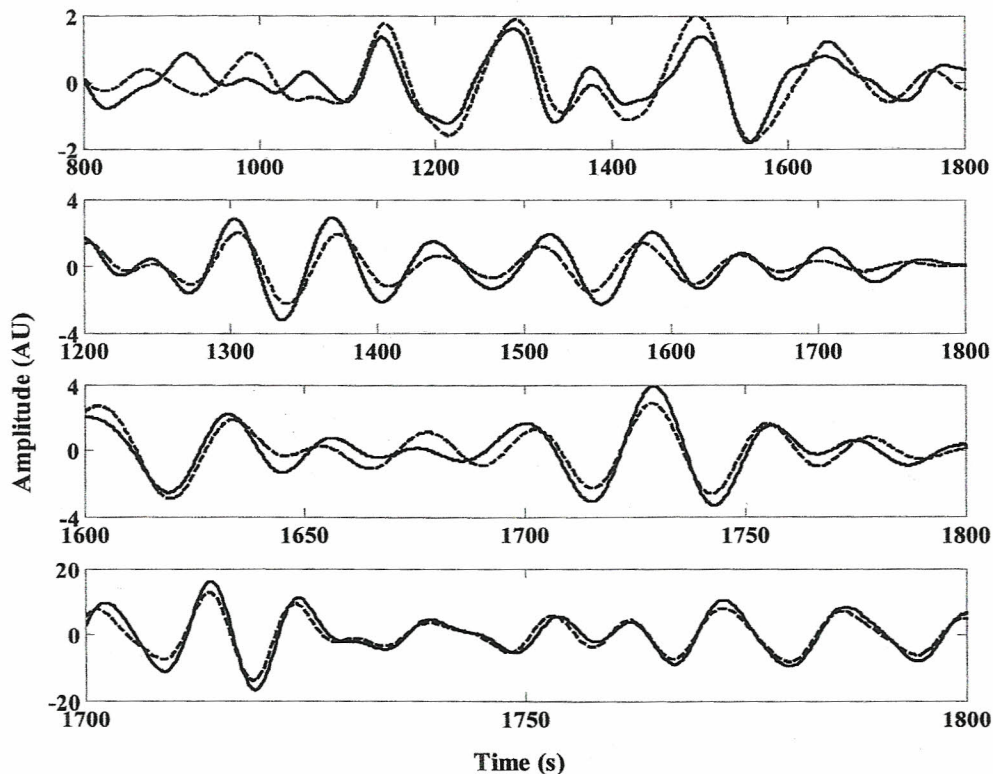
Figure 2: Filtered blood-flow signals in the bands B1 to B4 (top to bottom) from two adjacent regions (continuous and dashed lines) of the volar forearm of a volunteer.

The mean values and standard errors of the percentage of time of correlation (PTC) of the studied group (n=20), during basal temperatures (32 ^{0}C) and thermally stimulated (42 ^{0}C), are shown in the bar diagram in FIG. 3. Statistically significant ($p<0.05$, paired t-Student) increases of PTC were observed in the bands B2, B3 and B4 when thermally stimulated. No significance was found for B1. During heat stimuli, the observed mean values of PTC were from 80% to 87%, while ranged from 54% to 72% during basal temperatures (32 ^{0}C).

As thermal stimulus increased the PTC values, we compared the coefficient of variation (CV) of the parameter PTC with those from the mean amplitudes of the filtered signals in each studied band (B1 to B4), during thermal stimuli, as shown in FIG. 4. As we can verify, the CV values of the parameter PTC are lower than the respective CV values of the amplitudes for all bands.

In this study correlations between low-frequency fluctuations of flow signals from two adjacent regions of forearms, measured by the laser Doppler flowmetry, were assessed. No similar studies were found in literature. Thus it was not possible to compare the results obtained.

It was found that low-frequency fluctuations in flow signals, measured at two adjacent regions of the forearm of healthy volunteers, were correlated during long time intervals. This fact was verified not only during basal temperatures, but also during thermal stimuli, although during the latter condition, the studied paired signals remained longer time intervals correlated.

Studies of the relationship (correlation or coherence) of flow signals with other cardiovascular signals (ECG and blood pressure) are abundant in literature, since the correlation or coherence suggests a common origin, or dependency relationships between signals.

In general, correlation or coherence between two signals is not sufficient to assume causality (cause and effect relationship). It is unlikely, however, that two flow signals remain correlated for long time intervals and they are not related. Further studies are necessary to assign a common origin or any other relationship.
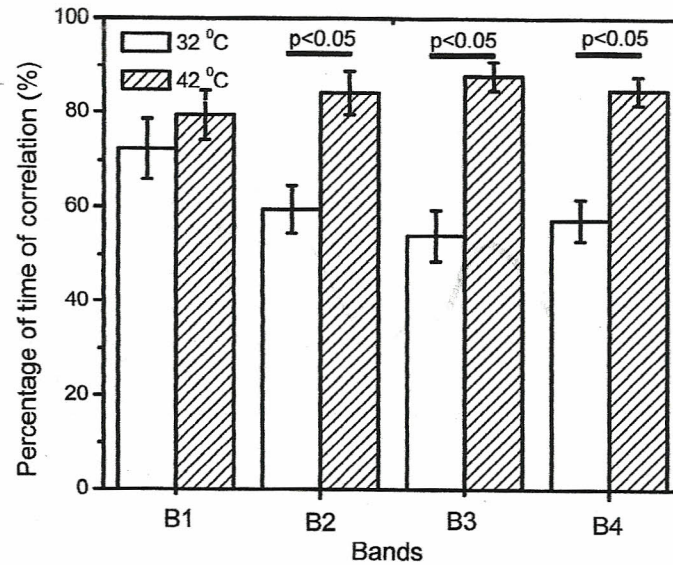

Figure 3: Mean values and standard errors of percentage of time of correlation between pairs of signals, from two adjacent regions of 20 volunteers, collected at 32 ^{0}C and thermally stimulated (42 ^{0}C).
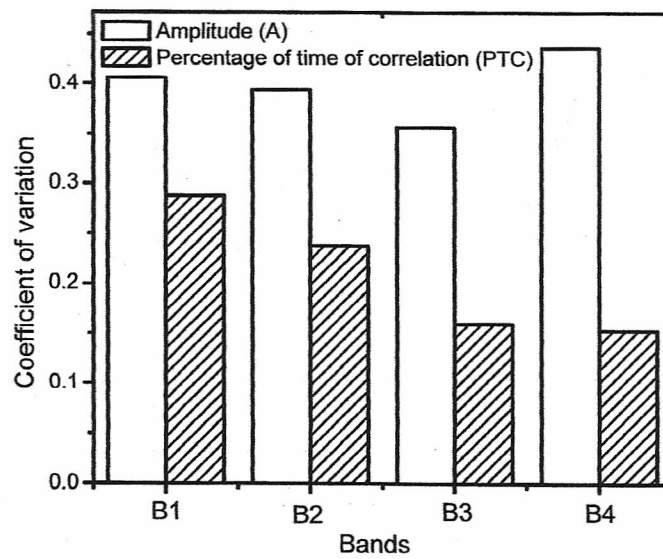

Figure 4: Coefficients of variation of the mean amplitudes (A) and of mean percentages of time of correlation (PTC) of blood-flow signals filtered in the frequency bands B1 to B4.

4. CONCLUSION

The results obtained indicated that low-frequency signals in the endothelial, endothelial-metabolic, neurogenic and myogenic corresponding bands, from two adjacent regions of volar forearms, when thermally stimulated (42^0C) remained correlated during most of the experimental period. Furthermore, the observed variabilities (as measured by the coefficient of variation) of the percentage of the time that signals remained correlated were lower than those for the signal amplitudes, suggesting a promising discriminatory parameter.

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