Laser speckle contrast imaging of blood flow from anesthetized mice: correcting drifts in measurements due to breathing movements

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ABSTRACT

Background: Laser speckle contrast imaging allows non-invasive assessment of cutaneous blood flow. Although the technique is attractive to measure a quantity related to the skin blood flow (SBF) in anesthetized animal models, movements from breathing can mask the SBF signal. As a consequence, the measurement is overestimated because a variable amount of a DC component due to the breathing movements is added to the SBF signal. Objective: To evaluate a method for estimating the background level of the SBF signal, rejecting artefacts from breathing. Methods: A baseline correction method used for accurate DNA sequencing was evaluated, based on estimating the background level of a signal in small temporal sliding-windows. The method was applied to evaluate a mouse model of hindlimb ischemia. SBF signals from hindlimbs of anesthetized C57BL/6 mice (n=13) were registered. The mean SBF (Fi and Fc from ischemic and control hindlimbs) were computed from the registers and from the corresponding estimated background levels (Fib and Fcb from ischemic and control hindlimbs). Results: The mean values of the percentages (a measure of ischemia) MI = (Fi/Fc).100 and MIb = (Fib/Fcb).100 were computed to be 30±4% and 23±3% respectively (mean ± SE). Evidences of statistical differences between both, ischemic and control hindlimbs, were obtained ($p<0.05$, paired student-t). The mean error [(MI-MIb)/MIb].100 obtained was 45±14% (mean±SE). Conclusion: The recovery of a corrupted SBF signal by breathing artefacts is feasible, allowing more accurate measurements.

Keywords: Laser speckle, blood flow, microcirculation

1. INTRODUCTION

Laser Speckle Contrast Imaging (LSCI) is a technique proposed to assess the microvascular function. Briefly, a low power (typically 50 mW), non-collimated laser radiation, frequently in the range from 635 nm to 780 nm, illuminates the skin. Scattered photons from static and moving (mainly red) blood cells in the microvascular plexus are collected by a CCD or a CMOS camera during a selected exposure time. The captured image presents a granular pattern, known as speckle pattern, due to interferences of the scattered radiation. The pattern changes with the movement of the red blood cells. The differences in the statistics of speckle patterns from static and moving structures are explored to compute a quantity related to the skin blood flow (SBF), here called blood flow. A sequence of images is captured, and each frame is processed producing a mapping of blood flow in the investigated region. A region of interest (ROI) can be selected to compute a regional (into the ROI) blood flow. The result is a time series of SBF values. This spatial processing methodology allows high sampling rate (typically in the order of 25 frames/s), detecting rapid changes of flow. As a result of the non-contact feature of the LSCI system, any body movement of the target generally interferes in the measurement [1]. This fact may restrict the use of the LSCI system to monitor the blood flow from anesthetized mice due to breathing movements. There is a great interest in monitoring blood flow changes in the hindlimb ischemia model in mice [2]. The aim of this paper is to evaluate a method for estimation of the SBF signal, rejecting artefacts from breathing.

2. MATERIAL AND METHODS

2.1 Model of hindlimb ischemia

Hindlimb ischemia was induced unilaterally in 13 male C57BL/6 mice (20 weeks old). Mice were anesthetized using Ketamine (50mg/kg) and Xylazine (10mg/kg) diluted in saline solution (0.9% NaCl). Femoral arteries were ligated

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at two distinct sites, immediately after and before epigastric artery, with silk sutures (5.0 Angiotech Pharmaceuticals, USA) as previously described [3]. The animals were kept in a temperature controlled facility. *Ad libitum* access to water and food with light and dark cycle of 12 hours were allowed. The animal care and protocols in this study were reviewed and approved by the Ethical Committee of Institute of Biomedical Sciences at University of Sao Paulo.

2.2 Laser speckle contrast imaging

Blood flow measurements were performed one day after the surgeries. Mice were anesthetized (described above) and hair was removed from both hindlimbs using a depilatory cream prior to the measurements of blood flow. The room temperature was maintained within 25±2^{o} C. Sunlight and other infrared sources of radiation were minimized during measurements. Blood flow measurements were made using a LSCI system, model moorFLPI Speckle Contrast Imager, Moor Instruments Ltd (Axminster, UK). The system uses a 50 mW laser emitting at 785 nm. The imager was positioned at a distance of 20 cm from the targets. In the image field, two rectangular ROI were selected covering the inferior hindlimbs, as depicted in Figure 1. The exposure time of the CCD was 4 ms. The mean blood flow from each ROI was computed using the acquiring software provided by the manufacturer. Low-pass filtering (time constant) was not used to smooth the register of blood flow. The sequences of mean values of blood flow (into the ROI), expressed in arbitrary units (AU), sampled at 25 frames/s, were stored in a computer. Registers were performed during typically five minutes. The calibration of LSCI was periodically verified according to the manufacturer instructions.

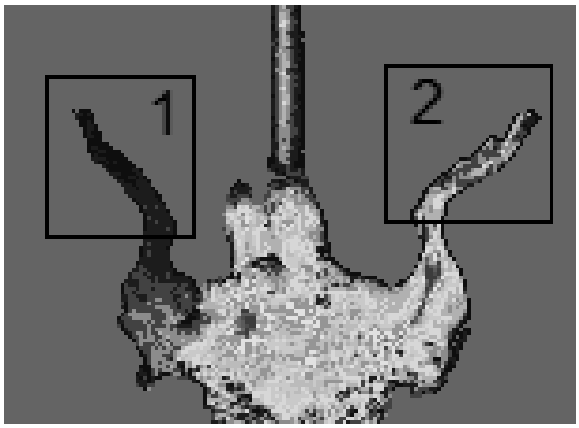

Figure 1. Typical mapping of blood flow from a C57BL/6 mouse produced via Laser speckle contrast imaging. Ischemic hindlimb in region (1) and control in region (2). Gray code: dark meaning low flow to white meaning high flow.

2.3 Signal processing

The (temporal) mean value of flow (Fi and Fc from ischemic and control hindlimbs) was computed from each register of blood flow and from the corresponding estimated background level (Fib and Fcb from ischemic and control hindlimbs). For this purpose, the mean value of each register was computed from the raw values of blood flow or from the estimated background level and then, the biological zero value was subtracted. The mean biological zero used (15 AU) was obtained from four mice, whose deaths occurred accidentally during the experiments. As a measure of ischemia, for each animal the percentage MI = (Fi/Fc).100 and MIb = (Fib/Fcb).100 was computed. The respiratory rate was measured via frequency analysis (Fast Fourier Transform - FFT) of the raw registers.

2.4 Background estimation

A normal breathing pattern is comprised by three distinct phases, namely inspiration followed by expiration and an automatic pause of almost no breathing. Figure 2 shows a register of a white light plethysmography from a mouse, evidencing the chest movement during the respiratory phases. During the inspiration and expiration phases, the movements of the lungs produce movements at the mice hindlimbs. As a result, the measured blood flow is corrupted by these movements. In contrast, during the pause phase there is no body movement and the measured blood flow is free

from artifacts due to breathing, here named as background signal. During the respiratory pauses (pause phase) the quantile of values of blood flow were automatically estimated using the MATLAB function msbackadj. The sliding-window and step size were set to be two and one seconds, respectively. Quantile estimation method was chosen (quantile equal 10%), and interpolation method used was pchip. In order to process longer signals, the code was modified to admit more than 200 separation units of windows.

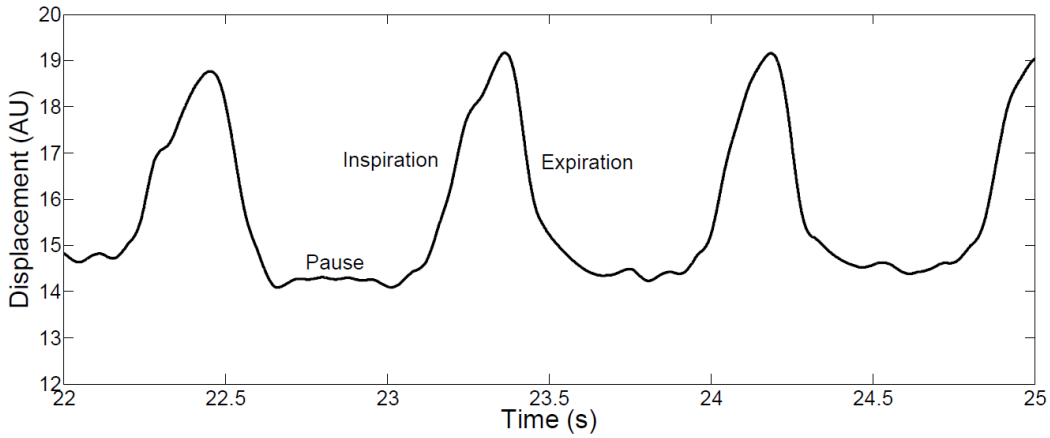

Figure 2. Plethysmographic signal related to a mouse chest movement during breathing.

3. RESULTS

A typical flow recording, during five minutes, and the corresponding estimated background signal from a control hindlimb is depicted in Figure 3. It is possible to see the pronounced oscillatory pattern due to breathing movements, represented here by peaks from 80 to 140 AU. The mean value of blood flow, computed from the raw register of blood flow (mean value from the five minutes register) and the estimated background signal, tracking the blood flow fluctuations, are also shown in this Figure.

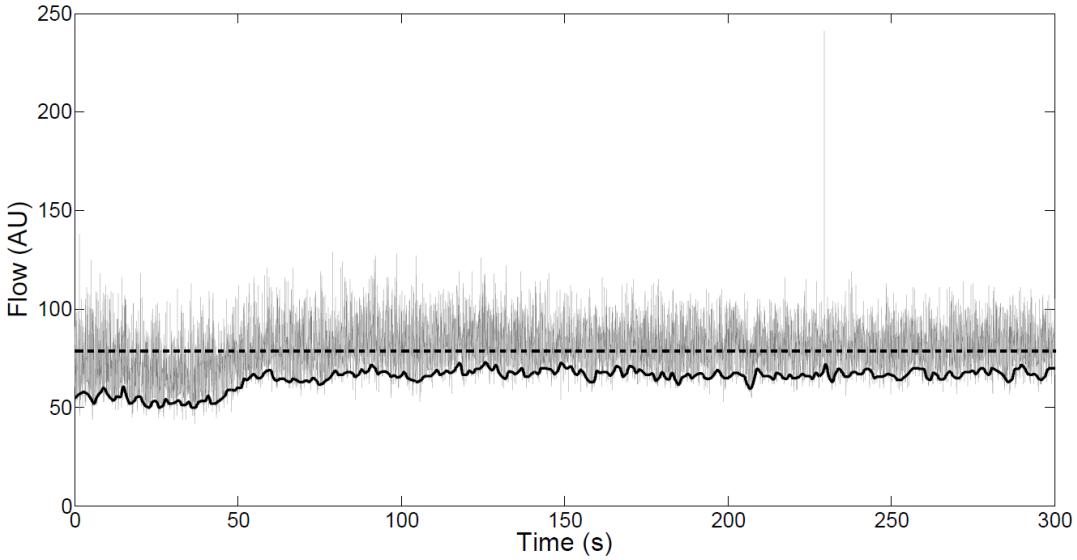

Figure 3. Raw register of blood flow from a control hindlimb during five minutes (gray), the corresponding mean value (dashed line) and the estimated background signal (solid line).

In Figure 4 it is shown a three seconds recording of an ischemic hindlimb blood flow (expanding the time-scale and upsampling the signal, allowing visualization of details). Peaks A and B represent to the inspiratory and expiratory phases, respectively, in which two maximal velocities occur (hindlimbs displacements during inspiration and expiration). The lower point between peaks A and B corresponds to the end of the inspiratory phase, when the direction of the movement of the hindlimb is inverted. In Figure 4 also are plotted the estimated background signal and the mean value of flow, computed from the raw register of blood flow. The mean value is clearly above the estimated background signal.

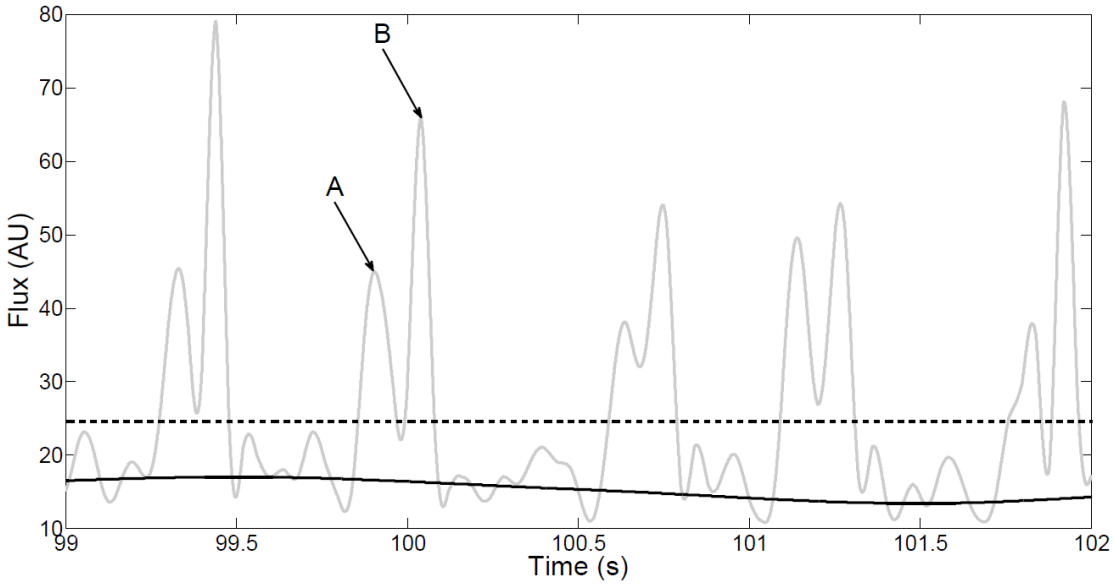

Figure 4. Raw register of blood flow from an ischemic hindlimb during three seconds (gray), the corresponding mean value (dashed line) and the estimated background signal (solid line). Peaks A and B represent to the inspiratory and expiratory phases, respectively, in which two maximal velocities occur.

Table 1 shows the values of percentage of ischemia for each animal derived from raw data (MI) and from the corresponding estimated background signals of blood flow (MIb), as well as the corresponding mean values. Significant statistical difference was obtained when comparing MI with MIb ($p<0.05$, paired student-t test).

In order to quantify the deviation of the percentage of ischemia derived from the raw registers and from the corresponding estimated background blood flow, it was computed the percentage of the error for each value of percentage of flow reduction. The observed values of errors ranged from 7.8% up to 136.1%, and a mean value of 45%.

4. DISCUSSION

The measurement of mice hindlimbs blood flow via LSCI is corrupted by respiratory movements. There are feasible strategies to overcome this problem. One of them, developed for the laser Doppler flowmetry, consists in visual selection of the pause phases and computing the corresponding mean values [4]. Because the blood flow in the microcirculatory system is a time varying quantity [5], a large amount of pause phases should be (visually) selected for a representative mean value. Thus, this is a time-consuming method. Other strategy could be to synchronize the capture of each speckle frame with the pause phase of breathing. Although modern LSCI systems are equipped with input for frame synchronization, this strategy requires an extra hardware to detect the breathing phases (generally unavailable to the common user).

In attempt to correct the baseline of a signal with peaks, the background signal of each register was estimated using the MATLAB function msbackadj. Baseline tracking and correction is a common problem in DNA sequencing.

Automated DNA sequencing is a well stablished technique. In this case, the accurate identification of a DNA sequence depends on the correction of the baseline of the chromatographic signal [6]. The raw chromatogram generally presents a slowly varying baseline. For DNA sequencing, the correction of the baseline is necessary in order to establish a trustable reference of the background signal to further processing. In this case, the corrected data is the raw data minus the estimated baseline signal. Here (blood flow measurement) the estimated baseline is the useful signal. The raw data are used only to estimate the background signal.

Table 1. Values of the percentage of ischemia computed from raw data (MI) and from the corresponding estimated background signal (MIb**).** [(MI-MIb)/MIb].100 (%) are relative errors. (*) Significant statistical difference ($p<0.05$, paired student-t test). SE (standard error).

Animal identification number	**MI(%)**	**MIb(%)**	**[(MI-MIb)/MIb].100 (%)**
#1	28.1	23.2	21.1
#2	12.4	6.9	79.7
#3	34.8	31.7	9.8
#4	48.2	44.7	7.8
#5	30	27.4	9.5
#6	29.3	21	39.5
#7	25.1	17.9	40.2
#8	38.4	34.2	12.3
#9	36.1	33.4	8.1
#10	10.1	3.9	159
#11	13.9	9	54.4
#12	57.6	24.4	136.1
#13	30	27.4	9.5
Mean±SE	**30±4**	**23±3**	**45±14**

There are two methods for baseline estimation implemented in the MATLAB function msbackadj. One of them is the quantile of the data. This kind of baseline corrector, among other popular methods, are based on the temporal segmentation of the raw data followed by the use of either their minimum, or mean, or a percentile of the data as an estimate of the signal background [6]. The second method is based on an Expectation-Maximization type of algorithm, assuming that every sample is independent and identically distributed, draw of any of two normally distributed classes (background or peaks), [6]. This method was not used here (produces similar results requiring more computational effort).

For the investigated mice, the respiratory frequencies (f) observed were from 2.4 Hz to 3.3 Hz (via FFT analysis). We observed experimentally (via plethysmography, data not included) the pause phase to be approximately 38% of the respiratory time interval (T), i. e., the inverse of the respiratory frequency (T=1/f). Thus, for the sampling rate used (25 frames/second), for each respiratory period T at least two samples were obtained during the pause phase (from 2 to 4 samples, depending on the respiratory frequency). The width of the sliding-window used was two seconds (50 frames). In this condition, for each window, the background estimation was performed using from 13 to 19 samples from the pause phase. Wider windows may improve the background estimation with the cost of a lower ability of signal tracking. It must be noticed that other mouse strain or even rats may present a (characteristic) lower respiratory rate. In such cases the width of the sliding-window must be readjusted.

Considering the background signal as a real blood flow signal, the mean values of blood flow computed ranged from 7.8% to 136.1% above the background level. Thus, the measured blood flow, under the experimental conditions mentioned above, is overestimated when background estimation is not performed.

5. CONCLUSION

The measured SBF signal from mice hindlimbs via LSCI, under the experimental conditions mentioned above, is corrupted by respiratory movements. The recovery of a SBF signal corrupted by artefacts from breathing is feasible, allowing more accurate measurements.

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