

Letter to the Editor

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Roustit et al (2010) investigate the reproducibility of Laser Doppler Flowmetry (LDF) as a means of determining cutaneous vascular conductance (CVC). As in other recent studies they seek to evaluate reproducibility by averaging relatively short time segments of data during or immediately after some perturbation. They conclude that the reproducibility of measurements on the forearm is limited by spatial variability in the microvasculature, even if the temperature and other relevant parameters are held constant. They have raised important questions that are apposite to the future use of LDF in clinical contexts, but we would like to contribute four comments to the discussion.

First, the reproducibility of forearm LDF measurements was investigated in earlier work (Bračič and Stefanovska, 1998). It was established that the issue of spatial variability could be mitigated by careful placement of the sensors: good reproducibility was obtained by avoiding proximity to the larger vessels, hairs, and blemishes. This was true both for spatial reproducibility, with simultaneous measurements at different positions on the same arm, and for temporal reproducibility, with sequential measurements at the same position. Note, however, that the criterion used for reproducibility did not involve the use of simple time averages.

Secondly, we would question whether time-averaging provides a satisfactory method for characterising blood flow, developing LDF criteria, or testing LDF reproducibility. Because blood flow is inherently oscillatory in nature (Bračič and Stefanovska, 1998; Aalkjaer et al. 2011), averaging will inevitably produce variable results depending on how the window is positioned relative to the phase of an oscillation unless, of course, the window is very much longer than the oscillation period. In reality, the situation is even more complex because there is not just one oscillatory process in blood flow, but at least six (Stefanovska, 2009). Fig. 1 shows a wavelet transform of typical LDF blood flow data. The slower of the two endothelial-related oscillations has a period of about 0.007 Hz, so that the averaging window would need to be much longer than 2.4 minutes in order to avoid irreproducibility from this source. The averaging intervals used by Roustit et al were actually 1 minute and 3 minutes, so that irreproducibility of their averages was to be expected.

Thirdly, we point out that the use of averages does not make best use of the information contained in LDF blood flow data. One can in principle always achieve reproducibility of an LDF average by using a long enough averaging interval, or by averaging over a large enough spatial area but, in doing so, one inevitably throws away a lot of potentially useful information. We would argue that it is better to accept that blood flow is inherently oscillatory, and to frame the criteria for LDF reproducibility on that basis. Thus, rather than asking whether the average blood flow has changed over time or in spatial position, it will be better and more rewarding to ask whether the characteristics of the oscillations have changed, for example: their amplitudes and frequencies, which are already known to be reproducible in time and space (Bračič and Stefanovska, 1998); or the extent to which the different oscillations mutually interact and perhaps synchronize with each other. Changes in these quantities have been related successfully to several different pathological conditions – e.g. congestive heart failure, hypertension and diabetes – as well as to other states of the body like e.g. exercise and anaesthesia. Even if averages could be measured reproducibly, they would do little to characterise or help diagnose these conditions.

To illustrate these points we present in Fig. 1 an example segment of an LDF signal (from a 30 minute baseline measurement made on the skin of the arm at rest) transformed by means of the Morlet wavelet transform, showing variability within each of the six frequency intervals (Stefanovska, 2007;

Shiogai et al, 2010). Low frequency variability is attributable to mechanisms that affect vascular diameter, such as neurogenic, NO-related and non-NO-related endothelially mediated processes. In Fig. 2 we show the same LDF segment and a series of time-averaged flux values made with different window sizes. If a short time is taken to “read” the value, the difference between readings can be as high as 60% of the baseline value. The longer the window is, the less variable the average value becomes. However, as shown in Fig. 1 there are distinct patterns in the variability that are missed if only the average is taken into account. Moreover, the patterns are visible on several different time-scales so that a relatively long recording time is needed to capture the dynamical properties of the blood perfusion signal.

Roustit et al. (2010) determine short-time average CVC values following a variety of perturbations (Post-occlusive Reactive Hyperaemia (PORH), Local Thermal Hyperaemia (LTH), room temperature randomisation and the Stroop colour mental test), applied either sequentially or simultaneously. The PORH and LTH effects are measured with 20 minutes or less of rest between tests.

Fourthly, therefore, we comment that, in a complex dynamical system such as the skin microvasculature, any perturbation is likely to involve nonlinear hysteresis effects. In Fig. 3 we show the results of a numerical simulation of just two coupled oscillatory processes subjected to repeated perturbation. We use bi-directionally-coupled limit-cycle oscillators (based on Poincaré oscillators), subject to external perturbations and weak noise:

$$\begin{aligned}\dot{x}_1 &= -\alpha_1(r_1 - a_1)x_1 - \omega_1(y_1 - \beta_1 r_1) - \epsilon_1 x_2 + \xi_1(t) \\ \dot{y}_1 &= -\alpha_1(r_1 - a_1)y_1 + \omega_1(x_1 - \beta_1 r_1) - \epsilon_1 y_2 + \xi_1(t) - s_1(t) - s_2(t),\end{aligned}\quad (1)$$

$$\begin{aligned}\dot{x}_2 &= -\alpha_2(r_2 - a_2)x_2 - \omega_2(y_2 - \beta_2 r_2) - \epsilon_2 x_1 + \xi_2(t) \\ \dot{y}_2 &= -\alpha_2(r_2 - a_2)y_2 + \omega_2(x_2 - \beta_2 r_2) - \epsilon_2 y_1 + \xi_2(t) - s_2(t),\end{aligned}\quad (2)$$

$$r_i = \sqrt{x_i^2 + y_i^2}.$$

The numerical simulation of (1),(2) was performed using a fourth-order Runge-Kutta method for stochastic integration, with $h = 0.001$ time step. The parameters were set to values appropriate for the cardiovascular system: cycle radii $a_1 = a_2 = 1$; frequencies $\omega_1 = 2\pi 0.1, \omega_2 = 2\pi 0.011$; couplings $\epsilon_1 = -0.01, \epsilon_2 = 0.001$; parameters for speed of convergence $\alpha_1 = 0.001, \alpha_2 = 0.1$ and parameters for the centre of rotation $\beta_1 = 0.4$ and $\beta_2 = 0.01$. The noise is white Gaussian, with zero mean $\langle \xi_i(t) \rangle = 0$ and correlation $\langle \xi_i(t) \xi_i(s) \rangle = D\delta(t - s)$, where D is the noise strength ($D_1 = D_2 = 0.003$). A long initial transient time (1000s) was discarded so that a stationary state was obtained. The external perturbations $s_1(t), s_2(t)$ are simple step signals, each with length $t = 200$ s and amplitudes $s_{1H} = s_{2H} = 0.2$, as presented on Fig. 1 (a).

For the first 200 seconds the first oscillator is unperturbed and its time-averages are around the baseline (except for small deviations due to weak noise and coupling). During the high value of $s_1(t)$ ($t=200$ s- 400 s) the first oscillator is perturbed and its time-averages are affected accordingly. It is evident that x_1 is then subject to the gradually decreasing after-effect of the perturbation. This transient period ($t=400$ - 700 s) appears because the oscillator needs a certain time to converge to its limit cycle. The length of the transient depends on the characteristics and the parameters of the oscillator. The associated time-averages are affected and the values are far from the baseline. A second perturbation for ($t=700$ s- 900 s) involves perturbing both of the oscillators with $s_2(t)$. Note that, during this period, the first oscillator is subject to the additional and indirect influence of the second oscillator, resulting in higher time-averages. After the second perturbation $s_2(t)$ finishes, the first oscillator is again left in perturbed state and only gradually returns towards its baseline value.

It is evident that transients in the oscillatory behaviour may persist for much longer than the timescale of the perturbation itself. Due to the coupled nature of the oscillatory processes, perturbing either oscillator results in the transient behaviour of both oscillators, leading to changes in the time-averaged values (which obscure the oscillations themselves). Repeated perturbations result in overlapping transient responses.

In conclusion, consideration of the oscillatory behaviour of the human microvasculature suggests that, to evaluate its state by LDF measurements:

1. Recordings must be made over an adequately long time period.
2. The variability, as well as the mean value, of the flux should be considered. It can be described in terms of the parameter values characterising the oscillations.
3. When subjecting the microvasculature to a perturbation, care should be taken to understand the role of oscillatory processes: short-time-average values may capture only a part of the transient physiological response.

References

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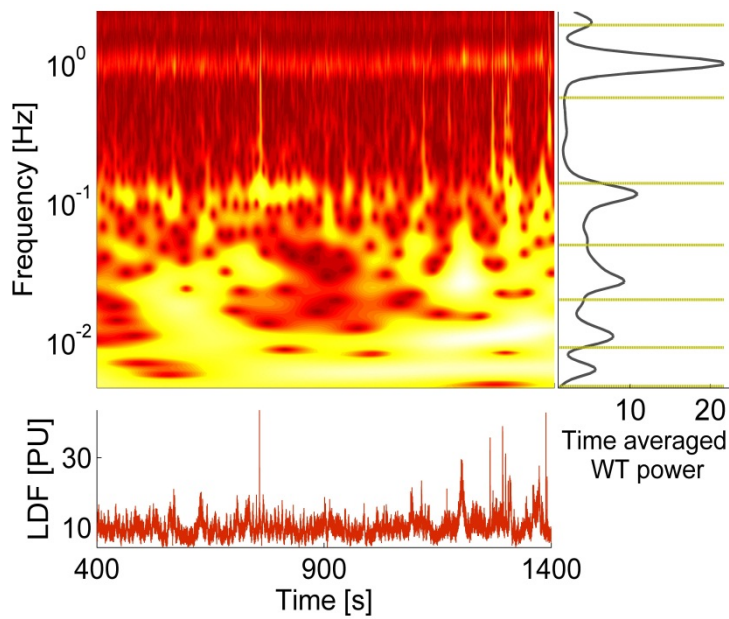


Fig. 1. Wavelet transform of LDF variability (top left), plotted above the raw signal in standard perfusion units (bottom) and the averaged wavelet power spectrum (right). The six frequency intervals described by Shiogai (2010) are indicated by horizontal lines and correspond (from the top) to: cardiac activity; respiration; myogenic oscillations; neurogenic oscillations; NO-related endothelial processes; and non-NO-related endothelial processes.

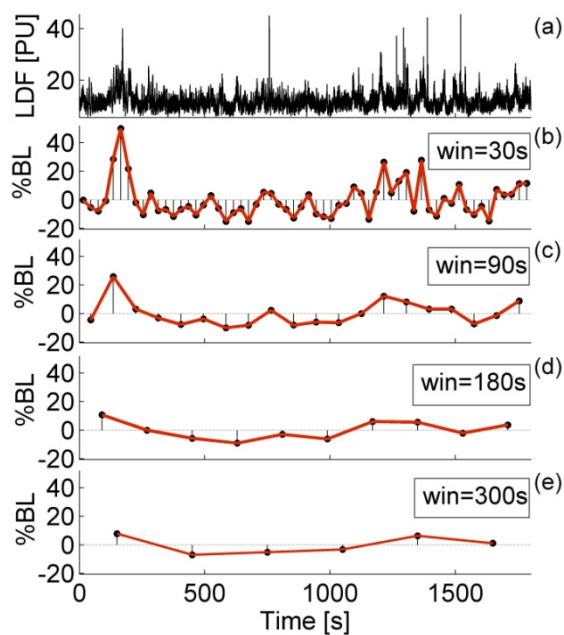


Fig. 2. The raw LDF signal from Fig. 1 averaged over successively larger window sizes, as indicated by the numbers in each box.

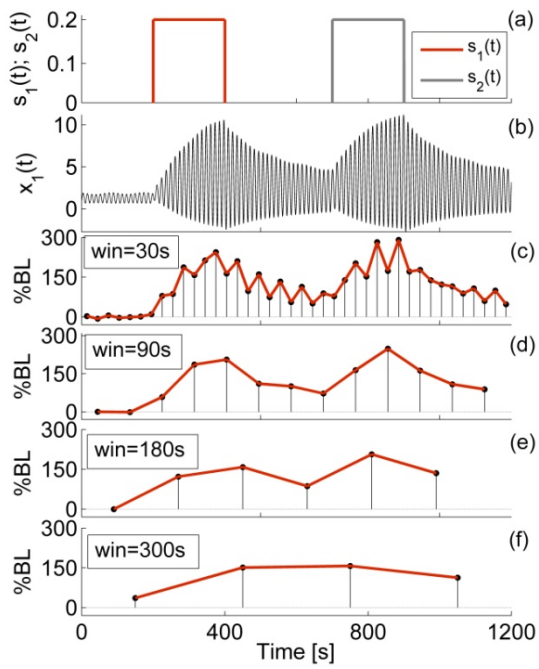


Figure 3: The effect of repeated perturbations on the two-oscillator model described by Eqs. (1),(2), showing the resultant changes in the mean value and transient effects as they present with different window sizes.