

Multi-scale, multi-domain analysis of microvascular flow dynamics

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New Findings

- **What is the topic of this review?**

We describe a range of techniques in the time, frequency and information domain and their application alone and together for the analysis of blood flux signals acquired using laser Doppler fluximetry.

- **What advances does it highlight?**

This review highlights the idea of using quantitative measures in different domains and scales to better mechanistically understand the complex behaviours in the microcirculation.

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Abstract

To date, time and frequency domain metrics of signals acquired through laser Doppler fluximetry have been unable to provide consistent and robust measures of the changes that occur in the microcirculation either in healthy individuals at rest, or in response to a provocation, or in patient cohorts. Recent studies have shown that in many disease states, such as metabolic and cardiovascular disease, there appears to be a reduction in the adaptive capabilities of the microvascular network and a consequent reduction in physiological information content. Here, we introduce nonlinear measures for assessing the information content of fluximetry signals and demonstrate how they can yield deeper understanding of network behaviour. In addition, we show how these methods may be adapted to accommodate the multiple time scales modulating blood flow and how they can be used in combination with time and frequency domain metrics to discriminate more effectively between the different mechanistic influences on network properties.

Introduction

Investigations of blood flow in microvascular networks have shown that in many disease states, such as cardiovascular and metabolic disease, there is a reduction in the adaptive capabilities of the network and its ability to respond to an imposed stressor (Frisbee *et al.*, 2016). The processes underlying the modulation of the blood flow are known to operate at different intensity levels and have different periodicities (Stefanovska *et al.*, 1999) which may both change temporally and spatially. Conventional time and frequency domain analysis techniques have proved valuable in the understanding of the network blood flow but have, so far, failed to describe mechanistically the changes in observed flow patterns between pathological conditions or haemodynamic states. Recently, nonlinear methods based on ideas from information theory have been used to quantify the regularity and randomness of short lengths of physiological signals and have demonstrated the potential for diagnostic capability (Balasubramanian & Nagaraj, 2016). These studies suggest that such methods may be of benefit in the analysis of microvascular network flow dynamics to discriminate better between different influences on network functionality and flexibility.

The aim of this brief report is to describe the application of a range of analysis techniques to signals derived from skin blood flow. We review the main applications and considerations of approaches in the time, frequency and complexity domains and their use in combination to discriminate between microcirculatory blood flow signals under differing pathophysiological and/or haemodynamic states. Examples are taken from two groups of individuals at risk of cardiovascular and metabolic disease that we have reported previously in detail elsewhere (Chipperfield *et al.*, 2019), grouped for the use (CB1, $n = 8$) or not (CB0, $n = 28$) of a calcium channel (CB) blocker for the treatment of hypertension. CBs are considered here as they are vasodilators that, in particular, inhibit myogenic control and can thus be expected to result in altered microvascular flow dynamics against which the different analysis methods may be tested.

Analysis in the time domain

Based on the Doppler effect, LDF was first described in 1964 for the measurement of polystyrene ball entrained in fluid (Yeh & Cummins, 1964) and first applied to retinal arteries and capillary tubes in 1972 (Riva *et al.*, 1972). The output signal is defined in arbitrary perfusion units (PU) as blood flux (BF) and is the product of red blood cell concentration and velocity. The volume of tissue that sampled is dependent on laser power, wavelength and separation between emitting and receiving fibres (Clough *et al.*, 2009) and is subject to significant spatial variations (Wahlberg & Fagrell, 1994).

Consider the two LDF signals shown in Figure 1(a) and (b) recorded from the forearm skin of two different people without and with CB use, respectively. The signal in Figure 1(a) shows a lower mean (8.3 AU) BF but with more variation in moving average than that in Figure 1(b) (mean 15.2 AU) which appears to have larger variation in the higher frequencies. Little information can be obtained directly from examination of LDF signals (Yvonne-Tee *et al.*, 2005; Roustit & Cracowski, 2012) alone as they provide a relative index of microvascular perfusion in the time domain. Thus, it is often used in conjunction with a vasoreactivity test, such as iontophoresis, local thermal warming or post occlusive reactive hyperaemia (shown in Figure 1(c)), to assess dilator capacity and mechanisms influencing vascular tone (Roustit & Cracowski, 2013). In the latter, resting BF (RF) is determined as the mean BF over some time period, typically 5 mins, before occlusion and the fold change MF/RF as the ratio of mean peak (MF) of the reactive hyperaemia. In the examples in Figure 1, the MF/RF values are 5.28 and 4.36 respectively indicating degraded dilatory capacity in the person with CB. A more robust test might account for differences in mean arterial pressure, e.g. (Ichinose *et*

al., 2019), to determine cutaneous vascular conductance when comparing groups although this requires additional measurements. While such analysis has been used in research and clinical practice, e.g. (Rossi *et al.*, 2008), its value as a diagnostic or prognostic indicator of vascular disease or pathogenesis remains disputed. As we have previously reported, reactive tests cannot reliably or sufficiently discriminate between different pathophysiological groups (Chipperfield *et al.*, 2019) alone or mechanistically in terms of vaso-control properties.

Analysis in the frequency domain

Analysis of BF time series reveals local, spontaneous, rhythmic oscillatory oscillation of BF (Figure 1) occurring at different frequencies which are taken to reflect the activity of local vaso-control mechanisms (Colantuoni *et al.*, 1994). These repetitive oscillations are taken to represent the influence of endothelial (0.0095-0.02 Hz), sympathetic (0.02-0.06 Hz), myogenic (0.06-0.15 Hz), respiratory (0.15-0.4 Hz) and cardiac activity (0.4-1.6 Hz) activity (Stefanovska *et al.*, 1999) and are usually made from resting BF signals. Two main methods are used to assess the spectral contribution of these component signals; one is based on the fast Fourier transform (FFT), the other on generalised wavelet analysis. The choice of method used has been discussed in detail elsewhere (Clough *et al.*, 2017) and is thus not considered further here. With the FFT, the power spectral density of the BF signal is obtained from its discrete Fourier transform in PU^2/Hz estimating the absolute power in the signal at a given frequency. Figure 2 shows the power spectrum, calculated using Welch's method, for the two individuals shown in Figure 1. The BF magnitude of the person with CB was previously observed to be larger and with apparently greater variation in the higher frequencies than the person without CB. However, examining the spectral content of the BF signals reveals that this is not the case with all bands having higher power without CB. Differences in mean BF shown in Figure 1 in PU are not seen in the power spectrum of Figure 2 as it does not contain the DC ($f = 0$ Hz) component and is shown in units of PU^2/Hz .

The relative power contribution of each frequency band is often used to evaluate its influence on the overall flow motion. In the case shown in Figure 2, large differences can be seen in all frequency bands. Much research has presented data from different pathological groups with known microvascular dysfunction (for examples, see review in (Clough *et al.*, 2017)) including responses to reactive tests. However, there is a lack of consensus on direction of change in oscillatory components of the BF signal and the balance between the absolute or relative power in the frequency bands with much appearing cohort specific and may

vary with measurement site (Clough *et al.*, 2017). Direct comparison of different studies is further complicated as the choice of parameters for frequency domain analysis (e.g. window size, overlap, number of bins) is a compromise between time and frequency resolution and signal parameters (e.g. sample rate, length and pre-filtering) may differ and methods can be sensitive to such parameter variations. Furthermore, recent work suggests that the frequency bands are not fixed and may vary, for example, with age or pathological state (Grinevich *et al.*, 2019). Nevertheless, such frequency domain analysis is a valuable tool in understanding the processes modulating BF and their relative contribution to overall flow.

Information and complexity

If the signals in Figure 1 could be accurately described by some mathematical model:

$$LDF(t) = f(t, x_1, x_2, \dots, x_n). \quad (1)$$

Then all the information in $LDF(t)$ would be described by the n parameters x_n and the original signal would effectively be compressed. However, if there was any error in the model then it would not describe $LDF(t)$ accurately and there would be a loss of information. The time and frequency domain analysis described previously lose, or doesn't use, all the information present in the LDF signals. The LDF signal is non-stationary as the processes that produce it vary in time and the power spectral density can only yield information of the overall signal content rather than the order or sequence in which it appears. Time domain methods generally describe mean values, or rates of change, and thus do not capture the variability in the signal. Attempts to model the data, e.g. (Tigno *et al.*, 2011), have shown relatively poor descriptive power.

Complexity analysis quantifies the degree of variability or loss of spontaneity in a time series and has been applied to a range of bio-signals, such as electroencephalograms (Kalev *et al.*, 2015) and electrocardiograms (Valenza *et al.*, 2017). The degree of variability in these signals reflect the physiological adaptability of the underlying system and are established biomarkers of overall health status (Aboy *et al.*, 2006). However, there is no single definition of complexity. Nagaraj and Balasubramanian (Nagaraj & Balasubramanian, 2017) describe three methods of quantifying complexity which here relate to (1) how hard it is to describe the information in $LDF(t)$, i.e. the number of unique patterns in the time series or model order, (2) how hard it is to create or losslessly compress the information and (3) the degree of organisation or structure in $LDF(t)$. The most widely used measure used in LDF signal analysis are Lempel-Ziv Complexity (LZC), sample entropy and effort-to-compress complexity

(Thanaj *et al.*, 2018). However, complexity is estimated, it provides a measure of the information content of a signal (Tigno *et al.*, 2011) and for brevity only LZC (Lempel & Ziv, 1976) will be considered here.

LZC provides a measure of how hard it is to describe the information contained in a signal and is the length of the shortest instruction set needed to reconstruct the signal without information loss. A simple periodic signal would have low complexity as the same terms are repeated continually. On the other hand, a random signal would have high complexity as there are no rules, or repeating patterns, that define it. As LZC does not require the signal to be stationary, unlike chaos-based entropy analysis, the complexity can be normalised to the length of sample window (Hu *et al.*, 2006). Before LZC can be calculated the original LDF signal must be transformed to a binary sequence which can be achieved by recording a one if a sample is greater than the median and zero otherwise (Albano *et al.*, 2008). Alternatively, a delta encoding method, whereby a zero is recoded if a value is less than the previous value in the time series or a one otherwise, which captures more of the variability in the signal can be used (Kuliga *et al.*, 2018). The signal is often divided in to epochs of suitable length to examine how LZC varies over time or a sliding window could be used to detect the time of rapid spontaneous changes in the signal.

Figure 3(a) shows an example of LZC calculated for 15×40 second epochs from forearm skin LDF captured at 40 Hz of the two different groups CB1, $n = 8$, and CB0, $n = 28$. As epochs are not synchronised in any way, direct comparison of individual or group values provides little understanding of spontaneous temporal activity. However, Figure 3(a) clearly shows differences in the amount of information contained in the BF signal, with the CB1 group being less variable and having fewer unique states (lower LZC) than CB0. An LZC-index, calculated here as the mean of the 15×40 second epochs, can also be used to examine the groups (Chipperfield *et al.*, 2019; Carey *et al.*, 2019). In this case, the LZC-index fell from 0.362 ± 0.05 in the CB0 group to 0.302 ± 0.05 (mean \pm SD) in CB1 with a significant difference between them ($p = 0.0013$).

Frequency, complexity and scale

The physiological processes that regulate flow-motion operate across multiple temporal scales ranging from 0.001Hz to 2Hz. and appear to vary with other parameters such as skin temperature (Kuliga *et al.*, 2018) and hypobaric hypoxia (Carey *et al.*, 2019). For the data presented above, the LZC-index correlates positively with dilator capacity (MF/RF) ($r = 0.47$, $p = 0.001$) and relative power in the respiratory band ($r = 0.52$, $p = 0.0001$) and negatively

with RF ($r = -0.37$, $p = 0.008$) and relative power in the cardiac band ($r = -0.56$, $p = 0.00004$) (Chipperfield *et al.*, 2019). The quite regular contribution to the information in the BF signal from the cardiac band, illustrated in Figure 2, appears to reduce the complexity of the CB1 group.

To account for these multiple, and potentially varying, process scales, LZC can be evaluated in multiple time-scales (MLZC) using a coarse-graining approach (Costa *et al.*, 2002; Cerutti *et al.*, 2009). The sampling frequency is altered by a scale factor τ defining the scale level used to resample the original signal reducing the scale of the time series. For the time series $\{x_1, \dots, x_N\}$, where N is the number of samples, the coarse-grained time series, y^τ , is:

$$y_i^\tau = \frac{1}{\tau} \sum_{j=(i-1)\tau+1}^{i\tau} x_j, 1 \leq i \leq N/\tau \quad (2)$$

That is, the LZC is evaluated at different LDF sample rates where, for an original sampling frequency of 40 Hz, the sampling frequency is $f_\tau = 40/\tau$ where τ is the scale factor. At scale $\tau = 1$ the original signal is preserved at 40 Hz and at scale $\tau = 24$ resampled to 1.67Hz. At scale factor one, the time series y^1 is the original signal and the length of each coarse-grained time series $\{y^\tau\}$ is equal to the original signal divided by the scale factor, τ . In previous work, we have investigated the length of signal required to obtain viable complexity measures and reported that a signal length > 1000 samples are required (Thanaj *et al.*, 2018) which equates to 10 minutes captured at 40Hz at scale $\tau = 24$.

Such multiscale analyses have been shown to be effective in understanding physiological signals in general (Costa *et al.*, 2002; Humeau *et al.*, 2010). Figure 3(b) shows an example of MLZC for the two groups CB0 and CB1. As scale length increases (lower frequency corresponding to higher scale), LZC can also be seen to increase together with the separation between the groups until the Nyquist frequency of the original BF signal is reached or passed. Assuming the maximum frequency of interest is the upper limit of the cardiac band of 1.6Hz, then the Nyquist frequency will be 3.2Hz. ($\tau = 12$). Above this scale, i.e. lower sample frequencies, the influence of the relatively periodic heart rate will be diminished and the signal contain more information. To reach higher scales would require longer BF recordings.

One advantage of MLZC over LZC is that it can provide more features for classification of LDF data from the complexity at each scale. For example, in (Chipperfield *et al.*, 2019) we

show that classification accuracy between these two groups can be improved from 77.8% using LZC to 86.1% with MLZC. A further benefit of MLZC is that it can be used to understand how the spectral components of the BF signal influence its complexity at different scales. For example, the Spearman's correlations between the power in each of the bands of the BF signals' power spectra and the MLZC at increasing scale for the CB1 and CB0 groups described previously are shown in Figure 4. Heart beat and respiration have significant but opposite correlations with complexity until the Nyquist frequency is passed where their influence reduces. Skin sympathetic nerve activity is known to be modulated by respiration and cutaneous vasoconstrictor neurones are temporarily coupled to cardiac and respiratory oscillations (Fatouleh & Macefield, 2013). Heart rate variability also contributes the complexity of the BF signal (Sassi *et al.*, 2015) and cardiac rhythm is modulated by respiration (Simms *et al.*, 2010). This coupling of the two high frequency components may partially explain why the MLZC increase with scale, especially as they exhibit little spontaneous variation under measurement conditions. At higher time-scales the resampled BF signal covers a longer time period and the lower frequencies associated with flow motion, that generally contain the majority of the signal's power, contribute proportionally more to signal variability resulting in higher complexity. The CB changes the magnitude and relative influence of all the power bands on the signal complexity with the cardiac pulse wave dominating.

Conclusions

Traditional time and frequency domain analysis methods alone are unable to provide robust and consistent descriptors of the microcirculation dynamics in either resting states or as a result of an imposed stressor. Further insight can be achieved through the combination of time, frequency and complexity domain analysis as illustrated here by the different combinations of processes modulation activity at different time scales between groups with and without CB uptake. The data presented here and elsewhere (Chipperfield *et al.*, 2019; Frisbee *et al.*, 2016; Tigno *et al.*, 2011) provide further evidence that attenuation of flow-motion patterns is associated with increased cardiovascular disease risk and prophylactic treatment results in a further decline of adaptivity through altered microvascular dynamics. The combination of techniques presented here open new possibilities for the analysis of signals arising from the microcirculation and elsewhere. The combination of, and relationship between, metrics derived in the different domains can provide robust parameters that account to some degree for the temporal scales of the signal's origin and underlying local and systemic activity. Together, this multiple domain analyses provide a platform to investigation of microvascular

impairment in the skin and disease risk which need to be applied to further pathophysiological states.

Author contributions

A.J.C., M.T. and G.F.C. all contributed to the writing of this review, approved the final version of the manuscript and agree to be accountable for all aspects of this work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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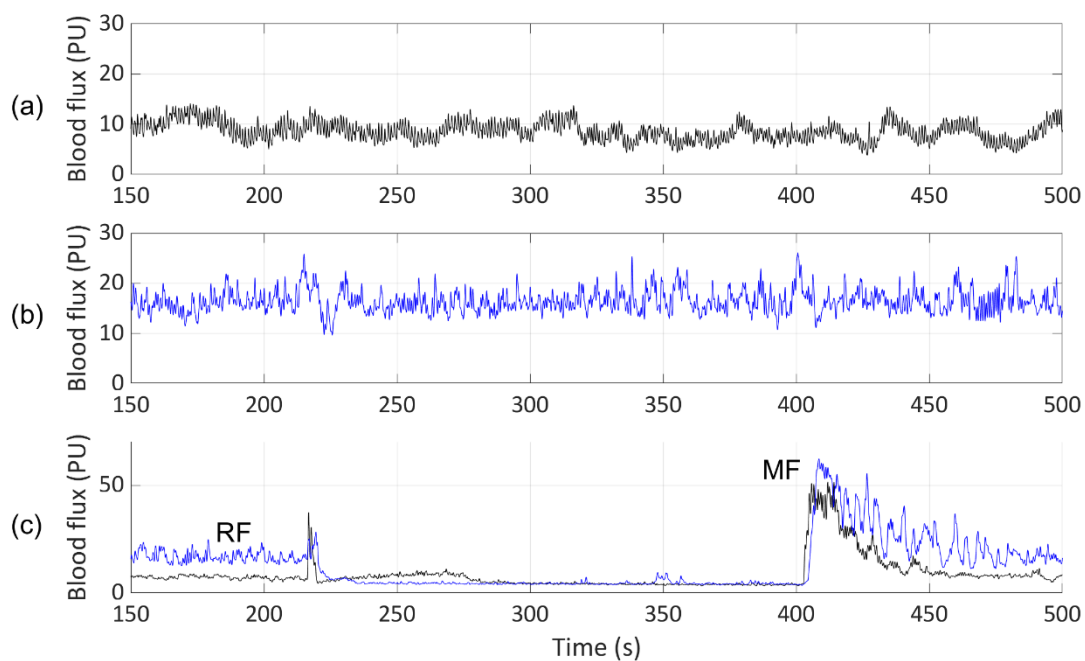


Figure 1: Examples of blood flux signals recorded from forearm skin at ambient room temperature in individuals at risk of cardiovascular and metabolic disease (a) individual without calcium channel blocker, (b) individual with calcium channel blocker and (c) at rest and during response to arterial occlusion (180mmHg for 3 min) (black without and blue with calcium channel blocker).

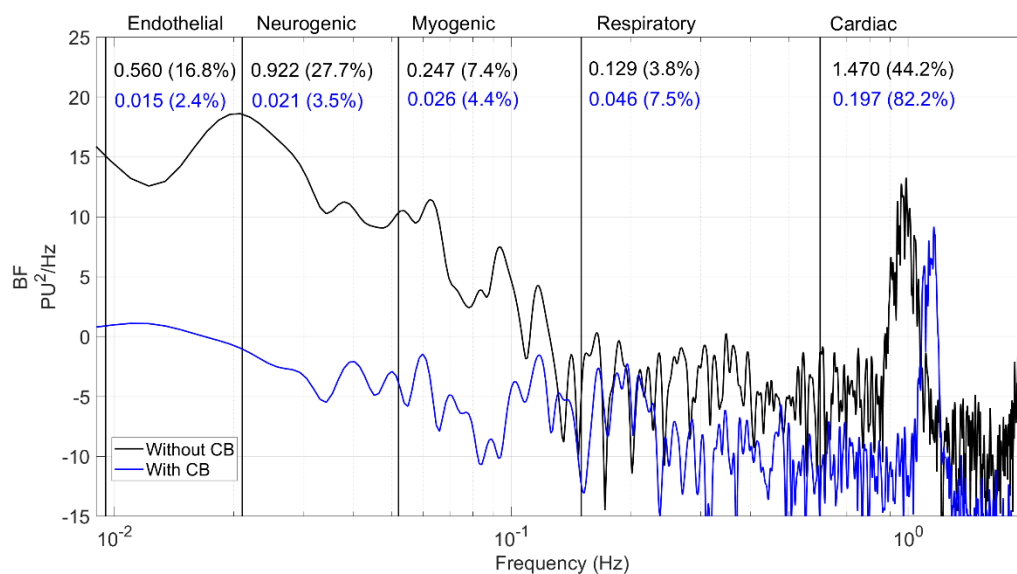


Figure 2: An example of absolute power spectral density plots for different individuals without (black) and with calcium channel blocker (CB) uptake (blue) showing mean and relative power per band.

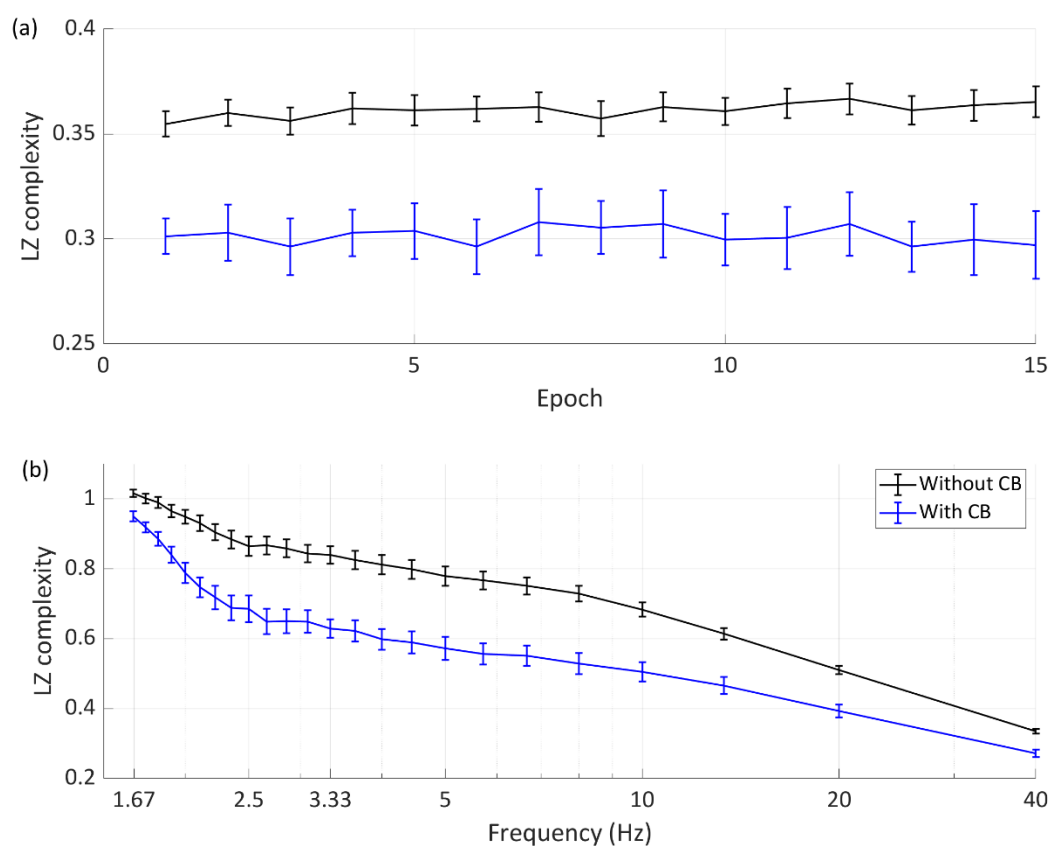


Figure 3: Examples of Lempel-Ziv complexity of forearm blood flux (a) calculated for 15×40 second epochs for forearm laser Doppler fluximetry signal captured at 40 Hz of two different groups of people at risk of cardiovascular and metabolic disease without (black, $n = 28$) and with (blues, $n = 8$) calcium channel blocker (CB) uptake and (b) at multiple scales (scale = $40/\text{frequency}$). Data are presented as mean \pm SEM.

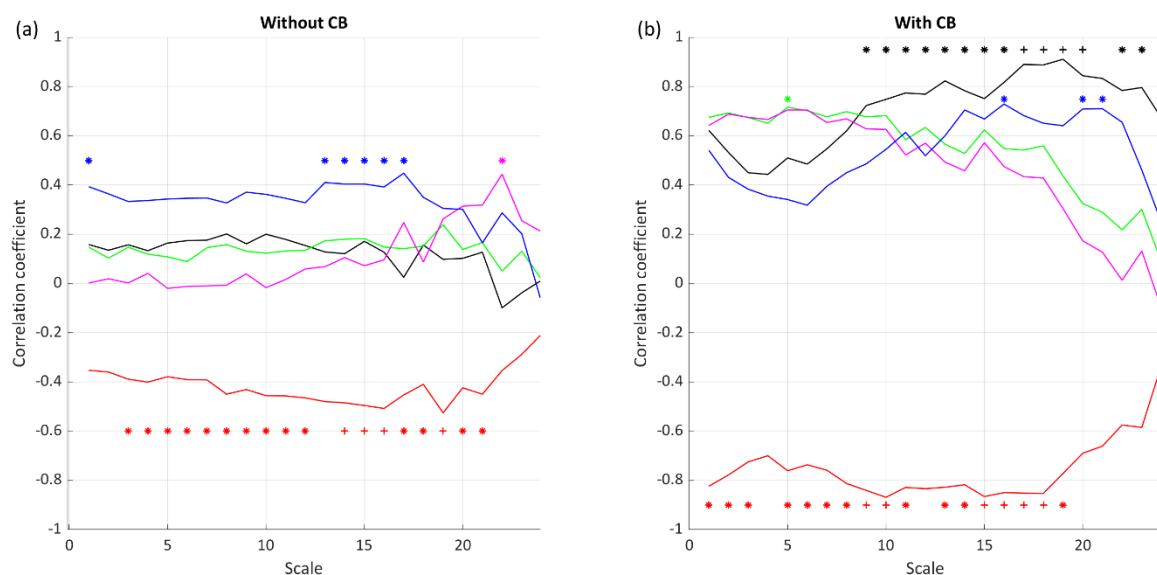


Figure 4: Spearman correlations between the flow-motion spectral power bands corresponding to endothelial (0.0095-0.02Hz) (black), neurogenic (0.02-0.06Hz) (green), myogenic (0.06-0.15Hz) (magenta), respiratory (0.015-0.4Hz) (blue) and cardiac (0.4-1.6Hz) (red) activity and multiscale Lempel-Ziv complexity of skin blood flow signals in (a) $n = 28$ people without CB and (b) $n = 8$ people with CB. Effective sampling rate = 40Hz/scale. * $p < 0.05$, + $p < 0.01$.