Laser Doppler Flowmetry in blood and lymph monitoring, technical aspects and analysis

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ABSTRACT

The aim of this work was to study the possibilities of the laser Doppler flowmetry method for the joint study of microhaemo- and lymph circulation of human skin.

Conducting a series of experimental studies allowed to trace the relationship of recorded signals of microcirculation of blood flow and lymph flow, as well as to study their oscillation nature by using wavelet analysis.

Keywords: blood flow, lymph flow, laser Doppler flowmetry, different frequency ranges.

1. INTRODUCTION

The primary method for liquid exchange in organic tissues is by diffusion and filtration mechanisms. Blood capillaries are the main source of fluid entry into the tissue, and while venules similarly transport proteins. Around 5-10 % of capillary-venular filtrate is transported from the tissue to the lymph, and about 2-4 litres of lymph per day is returned back into circulation.

The lymphatic system performs a number of vital functions in the human body - the return of water, proteins and other macromolecules in blood lymphocyte recirculation, removal of macromolecules and antigens from the body fluids, involved in the metabolism and purifying matrix, anti-defence, transport of fatty acids, fat-soluble vitamins and other food substances entering the intestinal lymphatic capillaries[1].

The lymphatic system is closely related to the venous system. It provides an additional drainage of organs, as well as the purification of tissue fluid. Thus, from a diagnostic point of view, it is most effective to carry out simultaneous investigations of microcirculatory blood flow and lymph flow.

Despite its importance, the lymphatic system is currently not fully studied due to the lack of quality of diagnostic methods suitable for non-invasive *in vivo* evaluation of the lymph circulation.

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In humans, there are very few *in vivo* measurements of microlymph dynamics parameters[2]. In the 1990s, a group of Swiss researchers studied lymph microcirculation in the skin of the distal region of the dorsal foot area using fluorescent video microscopy (microlymphography) after intradermal administration of staining agent dextran isothiocyanate[3]. Currently, a number of diagnostic methods are available in clinical practice to assess the vasculature, including computer and magnetic resonance tomography[4], Doppler ultrasound[5], various methods of microscopy[6, 7], optical coherence tomography[8, 9], fluorescence imaging techniques[10], and others[2]. All these methods have their limitations, preventing their use to adequately assess both blood flow and lymph flow. Use of ultrasound can determine the level of the main flow and assess haemodynamics, but microvascular changes with the help of ultrasound are not registered. Methods of computer and magnetic resonance imaging have the same flaw. In addition, radiopaque methods are restricted for regular monitoring as they are invasive, involve toxic contrast agents and deliver a radiation load to the patient. The use of microscopy techniques requires relatively delicate tissues for investigation. Methods of optical coherence tomography are highly sensitive to fluctuations in the study area, as well as have a small diagnostic volume. The method of fluorescence lymphography, which is quickly gaining in popularity, requires intradermal injection of a fluorescent dye, which significantly limits the diagnostic procedures and excludes this as a non-invasive approach.

The laser speckle and Doppler methods of measurements present themselves as modern and completely non-invasive, allowing to receive information in real time[11, 12]. Building devices that implement the methods of obtaining speckle images is non-trivial and costly. However, methods implementing Doppler spectroscopy using a fibre optic probe are often very informative and allow for analysis of a large set of different parameters, in particular, examination of the microvasculature oscillatory processes[13, 14].

The aim of this work was to study the possibilities of the laser Doppler flowmetry method for the joint study of microhaemo- and lymph circulation of human skin.

2. MATERIAL AND METHODS

A hypothesis stating that using the standard functional tests (breath holding, occlusion, etc.), results in changes occurring in the microcirculation which lead to a redistribution of the AC signals spectral power in different spectral ranges of the Doppler frequency shift[15], was suggested at the formulation stage of the problem. This allows for the non-invasive evaluation of the scattering particle velocity distribution in the diagnostic volume.

An experimental LDF device model featuring fully digital processing of received photocurrent was employed for this study. This enabled the recording of additional information about the distribution of the microcirculation index in a range of frequencies (Fig.1).

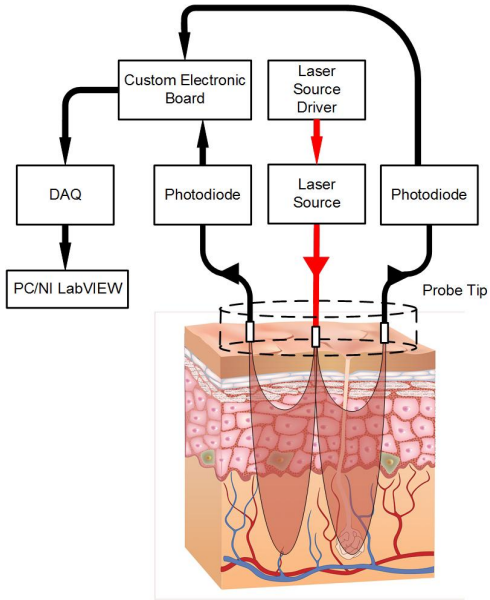


Figure 1. The general scheme of the measuring system.

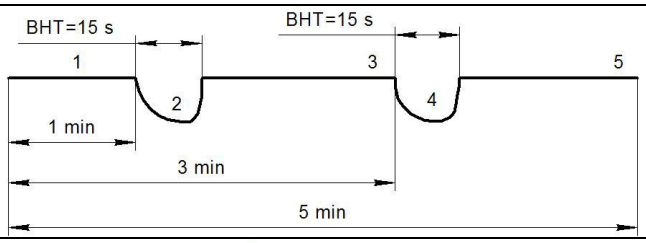
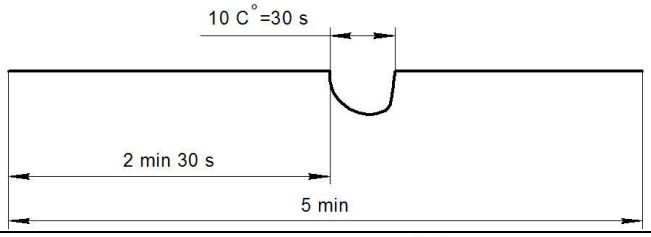
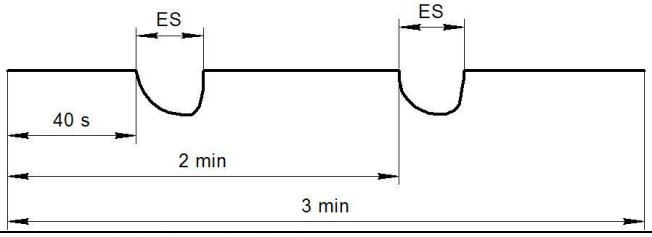
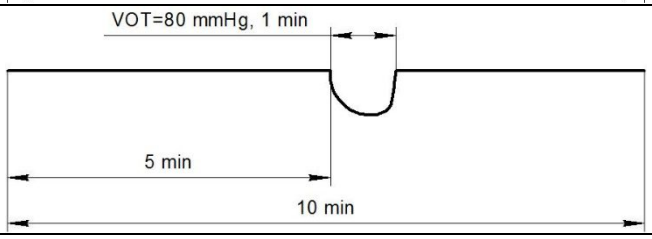
The radiation produced by a laser source with a wavelength of 1064 nm and scattered on the biological object was collected by a fibre optic probe and converted to photocurrent. The resulting signal was amplified and digitised on the NI USB 6211data acquisition board. The mathematical signal processing is performed on a personal computer in a programming environment NI LabVIEW using developed algorithms.

It has been suggested that, at lower frequencies, it is possible to record the movement of protein macromolecules in the lymphatic channel (lymphocytes themselves weakly scatter laser light due to their "transparency")[16].

The experimental part of the study involved a number of test measurements performed on healthy volunteers, simultaneously recording LDF-grams with the integration according to the following frequency bands: from 60 to 400 Hz, from 400 to 800 Hz, from 800 to 1600 Hz, from 1600 to 3200 Hz, from 3200 to 6400 Hz. This limitation of the frequency range is selected based on the fact that the signal strength at higher frequencies is negligible. Breath holding tests were recorded following a protocol involving sharp inhale-exhale breaths.

The study also used a modified LAKK series device (SPE "LAZMA" Ltd., Russia)[14, 17, 18]. Simultaneous recording of LDF-grams was carried out for two frequency bands: 300-10000 Hz – for registration of blood microcirculation; 20-200 Hz – for registration of lymph microcirculation. All recordings were taken from the palmar skin surface of the distal phalanx of the 3rd finger. We evaluate the index of microcirculation for different frequency bands in the probed field. To identify the relationship between blood flow and lymph flow using the LAKK series device, experimental studies on healthy volunteers (25 ± 3 years) utilising various functional tests were carried. The study protocol is shown in Table 1.

Table 1. The study protocols.

Functional Test	Number of Measurements, *n*	Study Protocol
Breath Holding Test, 15 s	22	
Temperature Test, 10, 35, 42 °C	3	
Electrostimulation Test, 5 mA	3	
Venous Occlusion Test, 80 mmHg	3	

Also, to the study the capabilities of the wavelet analysis, the vibration amplitudes of blood flow and lymph flow at the time of the venous occlusion test were determined. The test involved placing a blood pressure cuff around the arm and inflating it to mid-way between the systolic and diastolic blood pressures (~80 mmHg during 1 min). We studied the active frequency ranges (endothelial, NO-related activity, 0.0095-0.02 Hz; neurogenic sympathetic 0.02-0.046 Hz; myogenic 0.07-0.145 Hz) and passive frequency ranges (cardiac or heart 0.8-1.6 Hz, respiratory 0.2-0.4 Hz)[13, 14].

Wavelet analysis was also performed for lymph flow records. However, in this case the frequency boundaries 0,02-0,046 Hz correspond to the work of pacemakers in the lymphatic vessels[16].

3. EXPERIMENTAL RESULTS AND DISCUSSION

Multidirectional LDF-gram reactions at different frequency bands were observed during breath tests recorded by the experimental LDF model (Fig. 2). Increases in the low-frequency bands of the Doppler shift were observed during the local reduction of the overall signal (Fig. 2b). This effect was observed up to a frequency of about 800 Hz.

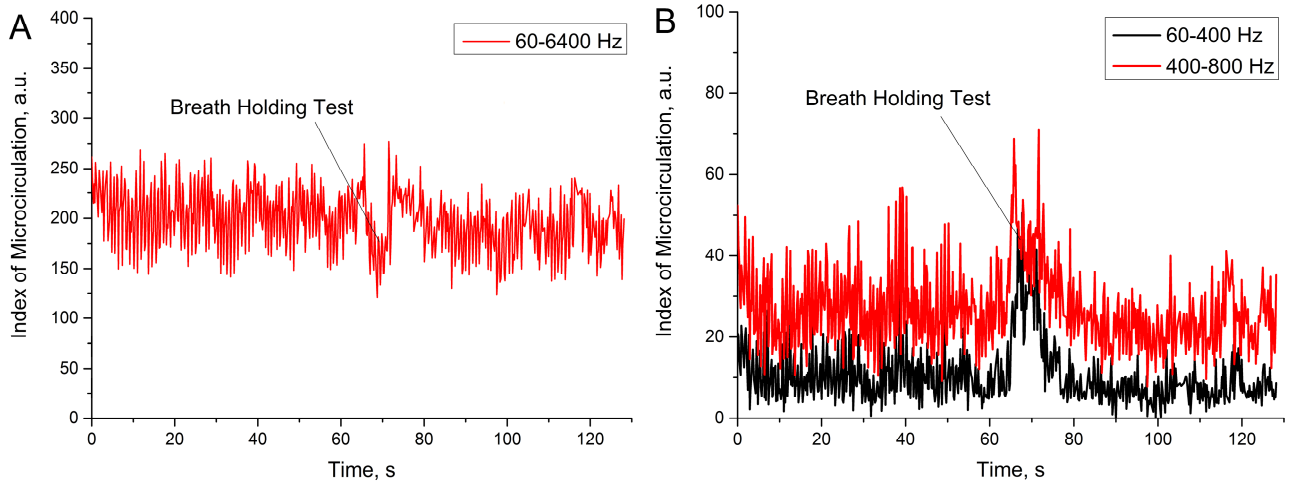


Figure 2. Example total LDF-gram of a breath test (a) and LDF-grams recorded using the experimental model on the 60-400 Hz and 400-800 Hz sub-band spectra (b).

An inverse correlation between microhaemo- and lymph circulation with Pearson's correlation coefficient r = -0.7 for both inhale and exhale was observed during breath tests with the LAKK device in all 22 studies (Fig. 4).

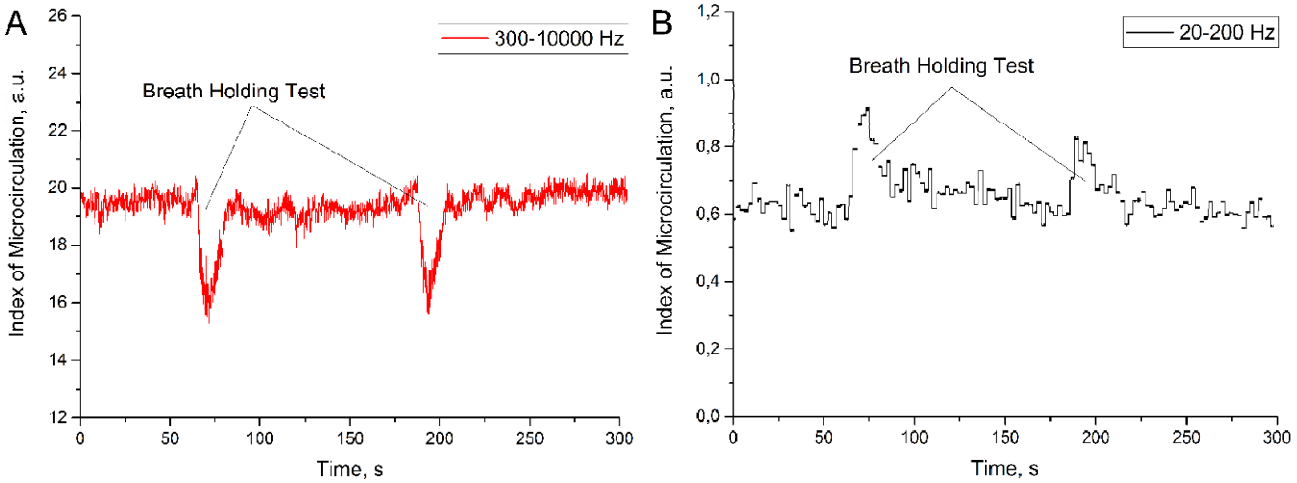


Figure 3. Example LDF-grams recorded with LAKK series device on the 300-10000 Hz (a) and 20-200 Hz (b) sub-bands of the spectrum during the breath test.

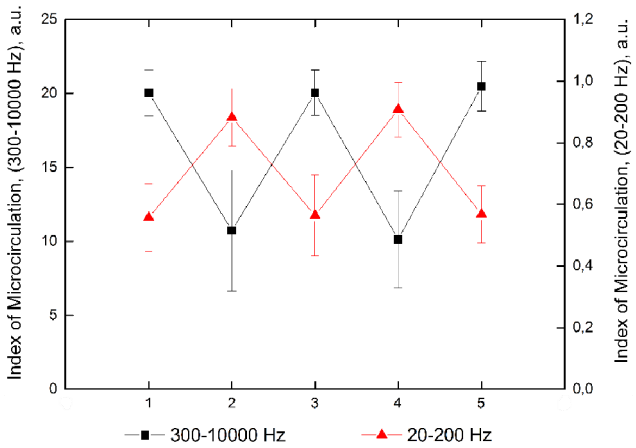


Figure 4. Relationship of microhaemo- and lymphocirculation during breath holding test.

Additionally, multidirectional reactions in the microcirculation at different frequency ranges were observed when conducting research with other functional tests (Fig. 5-Fig. 7).

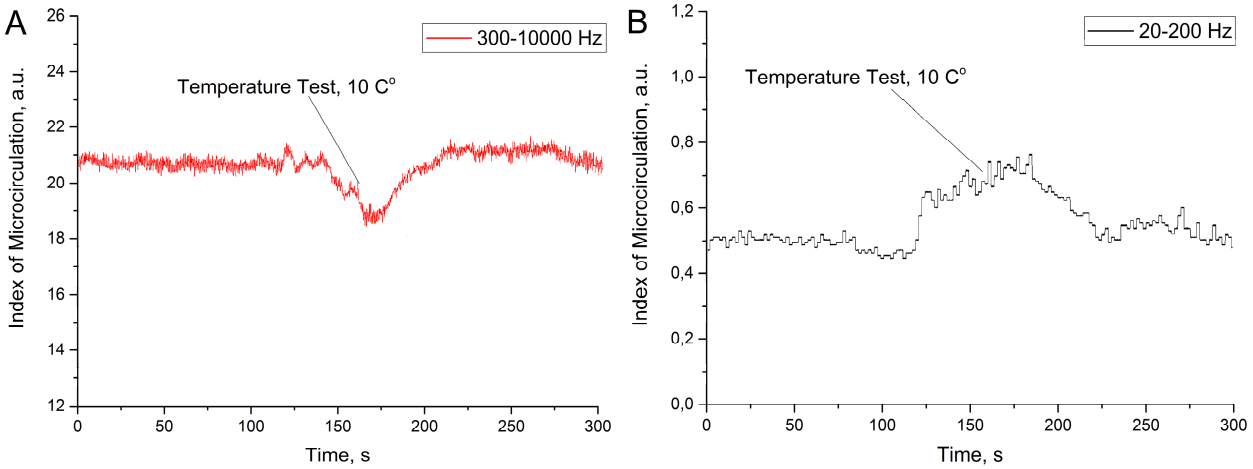


Figure 5. Example LDF-grams recorded with LAKK series device on the 300-10000 Hz (a) and 20-200 Hz (b) sub-bands of the spectrum during the temperature test.

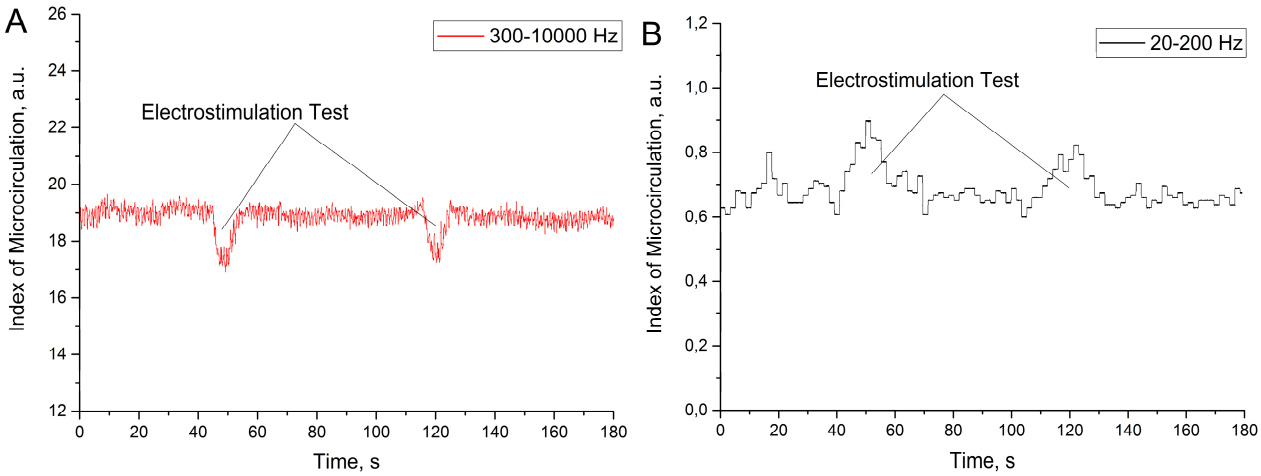


Figure 6. Example LDF-grams recorded with LAKK series device on the 300-10000 Hz (a) and 20-200 Hz (b) sub-bands of the spectrum during the electrostimulation test.

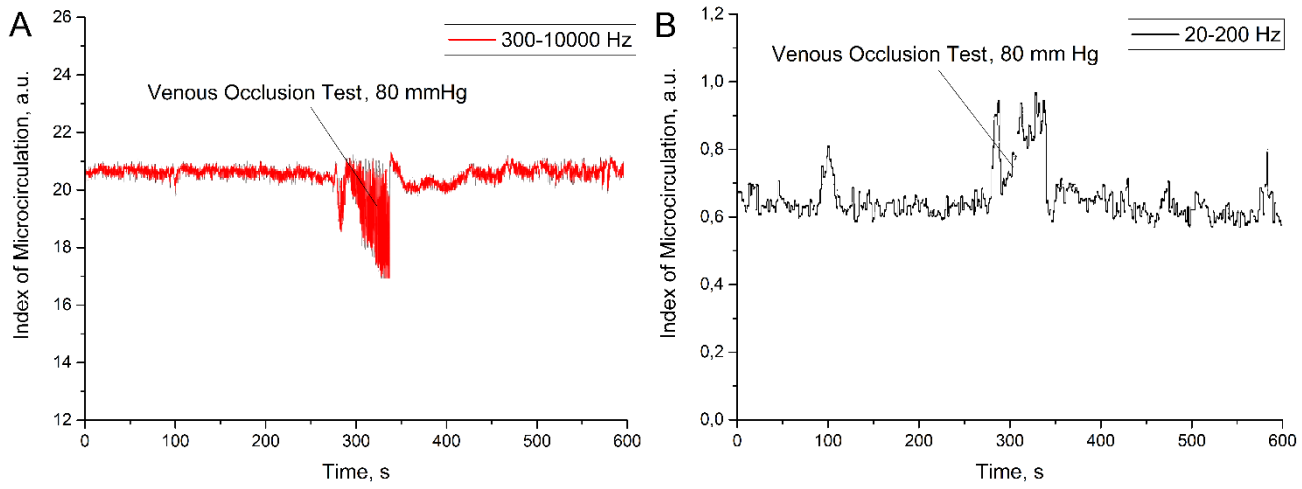
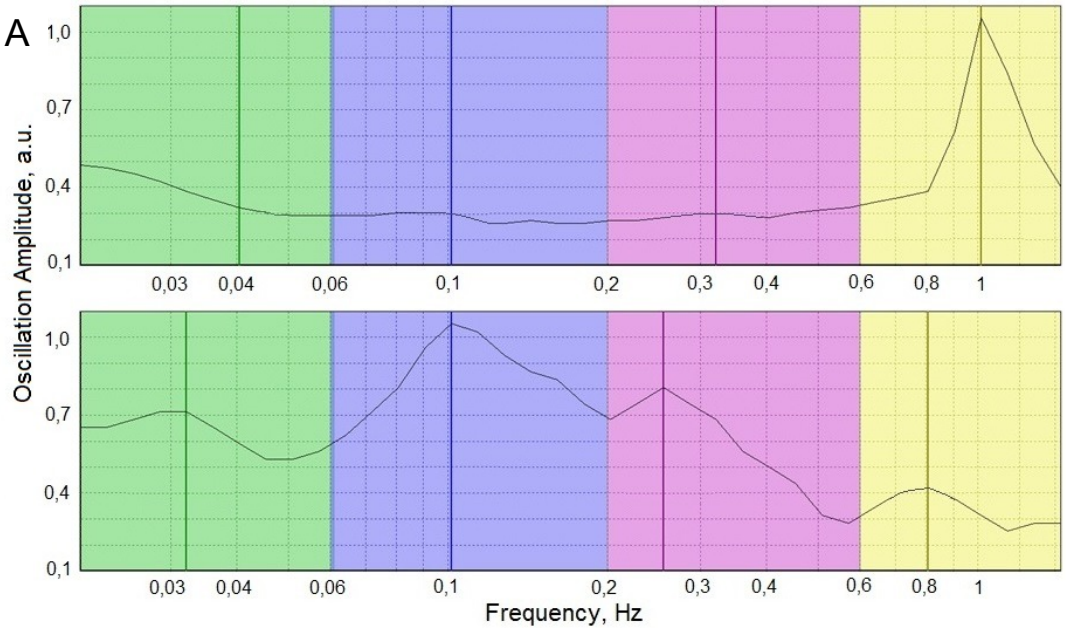


Figure 7. Example LDF-grams recorded with LAKK series device on the 300-10000 Hz (a) and 20-200 Hz (b) sub-bands of the spectrum during a venous occlusion test.

It should be noted that the observed pattern of LDF signal behaviour at functional loads can be associated with the number of reasons. Effect of microvascular spasms, leading to an increase in the number of slow-moving red blood cells and, as a result, increase in the microcirculation, localized in the low-frequency sub-ranges. In addition, during a spasm of arterioles and a reduction of distal perfusion pressure, a decrease in venular drainage activity is observed. This leads to an increase in pressure in the interstitium and an increased lymph flow.

The wavelet analysis results of the LDF signals recorded during venous occlusion showed that the oscillations of lymph flow in the microvasculature of human skin are characterised by distinct dominance of the pacemaker phase oscillations in the recovery period after the occlusion (Fig 8b), most likely transmitted from the deeper subcutaneous muscle-containing lymph vessels. In general, the wavelet analysis of the spectrum contained oscillations of other frequency ranges (myogenic and respiratory).



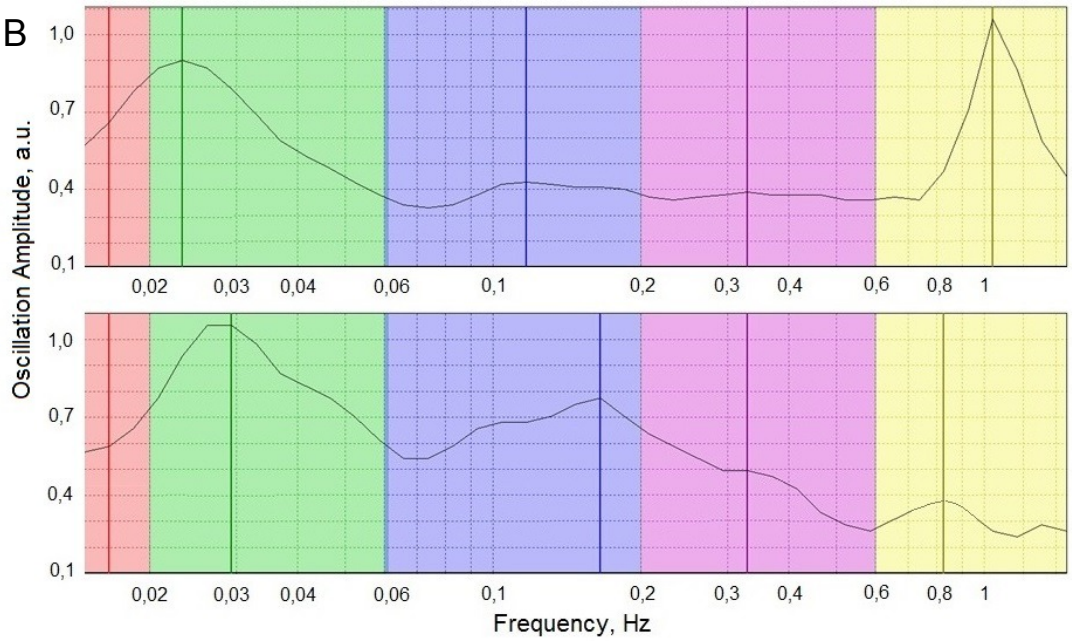


Figure 8. Wavelet-spectrum blood flow (the upper curve) and lymph flow (the lower curve) oscillations of palmar skin surface of the distal phalanx of the 3rd finger before the occlusion of the venous test (a) and in and recovery stage (b). From left to right - oscillation frequency (Hz): red area – endothelial rhythms (0.0095-0.02 Hz); green – neurogenic and pacemaker for lymph flow (0.02–0.046 Hz); blue – myogenic (0.07-0.145 Hz); purple – breathing (0.2-0.4 Hz) and yellow – heart (0.8-1.6 Hz).

Heart rate rhythms in the wavelet spectrum of skin lymph flow were not recorded since, apparently, due to anatomical reasons, they do not penetrate into lymph flow.

4. CONCLUSION

The proposed approach of Doppler measurements utilising multiple separate frequency bands revealed potentially useful results during joint investigation of the microhaemo- and lymphocirculation.

A high inverse correlation was observed in a joint analysis of the blood and lymph flow during a breath test. The results of the venous occlusion indicate that fluctuations in the flow of lymph in the human skin microvessels are supposedly characterised by the influence of increasingly pacemaker and myogenic oscillations. More low-amplitude oscillations were recorded in the respiratory range. Heart rate in the wavelet spectrum of lymph flow is not detected.

It should be noted that the movement of "slow" red blood cells and other blood protein molecules may contribute to the observed pattern of LDF signal behaviour. Separation of all the components of the sum signal is the main goal of further research. Also, the development of metrological support in this area is a very urgent task[19, 20]. Creating test facilities that simulate the movement of different fluids will allow a more detailed approach to the issue of separation of useful signals.

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