

Non-invasive optical monitoring of lower limb ischemia model

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A method of lower limb ischemia monitoring via photoplethysmography and videocapillaroscopy is proposed. Critical lower limb ischemia was modeled on lab rats via embolization of arteria femoralis and verified using magnetic resonance angiography and perfusion analysis. Experimental setup and a pipeline for investigation are proposed. Quantitative parameters for ischemic limb's condition assessment are proposed; the parameters represent changes in skin blood perfusion and capillary network density. A decrease of these parameters' values in ischemic limb is presented. Data acquisition parameters, as well as parameters that present significant differences before embolization and one day after, high sensitivity and specificity, are described. Acquired results compliment conventional methods of ischemia assessment with data on skin microcirculation. The approach proposed provides robust data acquisition, non-invasive measurements and easy-to-implement hardware and software. In the future it can become a highly demanded supplement to conventional methods of ischemia diagnosis and quantitative assessment, also during prolonged experiments involving various therapeutic scenarios.

Keywords: critical lower limb ischemia, non-invasive diagnostics, photoplethysmography, videocapillaroscopy, digital image processing, model animals.

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Introduction

Critical lower limb ischemia (CLLI) is a long-standing (more than 2 weeks) clinical condition characterized by obstruction of the main arteries of a lower limb with pain at rest and/or trophic tissue defect in the lower limb. Possible causes of CLLI progression include diseases and malconditions leading to obstruction of the lower limb arteries: atherosclerosis, thrombembolia, thrombosis, arteriitis, aneurysmal dilatation of artery, restenosis and thrombotic complications of reconstructions on limb arteries for arterial deficiency. Obliterative arterial diseases of the lower limbs occupy a large part among the CLLI progression causes, the main statistically relevant hazards include smoking, diabetes, arterial hypertension and hypercholesterolemia [1,2].

Critical lower limb ischemia corresponds to Stage III and IV of arterial deficiency of the lower limb according to the Pokrovsky-Fontaine classification and includes patients with pains at rest and with trophic changes, including secondary infectious and inflammatory changes in limb tissues [3].

Among CLLI diagnostic techniques, instrumental methods of examination are most informative. Duplex sonography is the primary vascular imaging method in patients with clinical signs of CLLI [4–6]. Information about the patient's microcirculatory bloodstream state is also obtained using transcutaneous oximetry [7], three-phase scintigraphy [8,9], computed tomography (CT) angiography [10–12], direct contrast angiography and magnetic resonance (MR) angiography and perfusion [13–16] that feature high sensitivity

and specificity. These techniques are particularly effective at late disease stages where tissues have already undergone irreversible changes [17]. Amputation of affected limbs is the only method of treatment at late disease stages [2,18,19]. Development of an ischemia screening procedure (including experimental one) for early stages where pronounced damage hasn't occurred yet and medication-based treatment is still effective is of scientific and practical interest.

Occurrence of ischemia in a limb is known to affect blood circulation not only in the main vessels, but also in the microcirculatory bloodstream [20–24]. Therefore, soft tissue perfusion evaluation can be an effective supplement to already existing methods allowing in-depth study of limb perfusion [23]. Moreover, numerical nature of some soft tissue perfusion evaluation methods expands the capabilities for diagnosing microcirculatory disorders over time and treatment efficacy [25,26].

Optical methods, the key benefit of which is in quick non-invasive examination, are widely used for microcirculation parameter evaluation [27,28]. Due to minimized intervention into the body, examination results are more reliable and analysis can be performed regularly for a long period of time. Such methods include photoplethysmography and videocapillaroscopy based on the analysis of time and spatial distribution of back-scattered radiation intensity vs. the state of blood components in capillaries [29–31]. Detection of time-varying back-scattered radiation intensity provides a photoplethysmogram (PPG) signal proportional to temporal

tissue blood filling fluctuations. Pixel-by-pixel analysis of such signals detected with high time and space resolution is used for imaging of capillaries characterized by high pixel brightness fluctuation amplitude on the series of skin area images due to scattered radiation intensity changes over time caused by blood particles travelling in the capillaries, i.e. for videocapillaroscopy [32–34].

This study is dedicated to the investigation of photoplethysmography and videocapillaroscopy performance for evaluation of blood microcirculation during experimental CLLI simulation in small laboratory animals and to the establishment of a data and parameter acquisition protocol to ensure analysis reliability.

Materials and methods

Experiment protocol

Experiment protocol is shown in Figure 1. The main phases included animal selection and preparation, acquisition of input limb perfusion data, critical ischemia simulation and acquisition of data on perfusion in animal limbs 24 hours after ischemization.

Experimental animals and ischemia simulation

Female laboratory rats ($N = 10$) were used as a model for the study. Critical ischemia was simulated in the right leg via femoral artery embolization. Before the experiment, hair on the leg skin was removed by depilation. A cut was made on the femur skin in the inguinal fold projection, soft tissues were moved apart by blunt dissection and the femoral vascular bundle was exposed. The femoral artery was exposed and ligated, a 0.3–0.4 mm cut was made in the front wall below the ligature, and a microcatheter was introduced through the cut into the vessel. Using a 1 ml syringe connected to the catheter, an embolic suspension was introduced into the artery and consisted of polymer microparticles with a diameter of 200–220 μm in swollen state, which resulted in perfusion termination in the embolized artery perfusion area. After this, the wound was sutured and treated with an antiseptic solution.

During embolization and perfusion data acquisition, the animal was under inhalation isoflurane anesthesia.

Experimental setup

A test bench setup for blood flow data acquisition is shown in Fig. 2. It consisted of a trinocular microscope ($2\times$ magnification), CMOS video camera (resolution 2048×1536 pixel size 3.45 μm , 12-bit A/D converter, frame rate 120 Hz) and green LED light source ($\lambda = 520 \text{ nm}$, 10 W). To reduce the contribution of the directly reflected component to the detected signal, the test area was coated with immersion fluid (paraffin oil) and covered with a slide 20 mm in diameter. The slide was used

to align the object surface which was necessary to provide a uniformly sharp image throughout the field of view ($2.5 \times 2.5 \text{ mm}$) at a shallow depth of field. The light source was energized from a programmable laboratory source with current remaining unchanged for all tests (0.7 A).

Data acquisition

Perfusion data acquisition was performed for each animal via photoplethysmography and capillaroscopy in two legs. A leg was nominally divided into three test areas (Figure 2, left): ankle (blue), knee (green) and hip (red). For each of the test areas, three zones were selected within the slide, and a sequence of 1500 12-bit frames with a frequency of 100 Hz was recorded for each of these zones. MR angiography was carried out for all animals to verify the artery embolism.

Data processing

Pre-processing of the captured image sequences used the algorithms that had been developed earlier by the authors [35–38]. At the first stage, spatial nonuniformity of illumination was corrected — each frame was smoothed using the Gauss filter and thus obtained low-frequency component was subtracted from the initial frame. Then the image contrast was increased due to the linear expansion of pixel intensity histogram of each frame. The next stage involved compensation of interframe object movements — a transformation matrix necessary for superimposing the frame with the first one was determined for each sequence frame using the previously proposed correlation algorithm [39]. The obtained matrices were applied to the image sequence obtained after the illumination nonuniformity correction. A set of images with minimized spatial illumination nonuniformity and motion artifacts resulted from preprocessing [36].

The quantitative microcirculation parameters were calculated. Time signal obtained by averaging the pixel intensities for each sequence frame was determined. The signal underwent frequency filtration in the 2.5–7 Hz range to exclude a component associated with oxygenation change and the high-frequency noise effect, and normalization to the mean signal before filtration to consider individual features of optical properties of tissues. The obtained normalized filtered signal represented a photoplethysmogram (PPG) describing blood filling with time. For further analysis, mean standard deviation of PPG (PPG, arb.units) proportional to the mean blood filling change amplitude of the test tissue area was calculated for each frame sequence. Standard deviation of PPG after normalization used as a quantitative blood filling metric makes it possible to perform the analysis of changes associated only with blood amount fluctuations in the test tissue volume due to the heart activity and minimizes the effect of individual skin features.

Density of active vessels (AVD, arb.units) was another calculated microcirculation parameter. To determine the microcirculation value, the PPG signal was first obtained

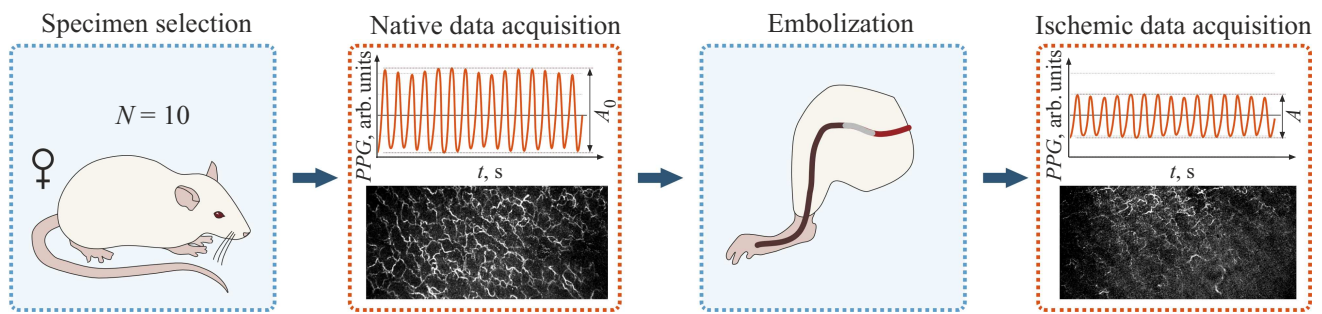


Figure 1. Experiment protocol

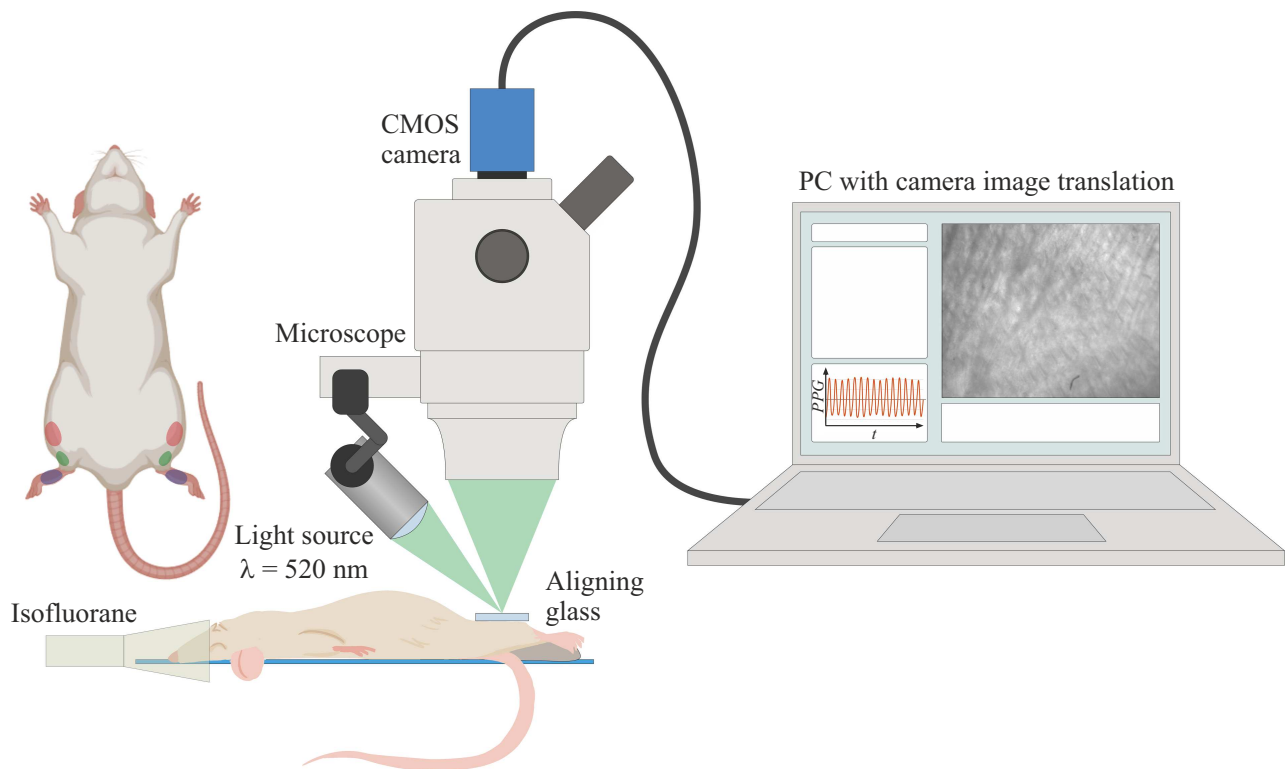


Figure 2. Experimental setup. Test areas are marked in different colors on the rat image: ankles (blue), knees (green), hips (red). Software providing translation of captured test region images (right) and PPG curve (left) are illustrated on the PC screen.

in each pixel from the superimposed frame sequence and pixel-by-pixel calculation of the PPG standard deviation was performed. For image areas pertaining to active vessels, i.e. vessels via which blood particle aggregates travel, significant pixel intensity fluctuations and, consequently, PPG amplitude fluctuations were observed. In other image areas, the signal amplitude was low and corresponded to the noise level. During further pixel-by-pixel calculation of the PPG standard deviation, much higher values corresponded to active vessel areas than to surrounding tissues, which ensured their visualization in the form of white lines against a dark background. Spatial distribution of the PPG standard deviation characterizes the vascular tree projection on the light receiver plane of the data acquisition system and is referred to as the vessel map. Density of active vessels was

obtained after vessel map binarization by a global threshold determined using Otsu's method and was expressed in white pixel percentage with respect to all image pixels.

Since the study involved 10 animals whose legs were measured in three zones within three areas, 30 PPG and AVD values each for each area and 90 PPG and AVD values each for each leg were obtained on each test day. PPG and AVD values were averaged over three zones within one leg area ($PPG_{\text{mean, area}}$ and $AVD_{\text{mean, area}}$, i.e. 3 values each per area) and over all leg areas ($PPG_{\text{mean, leg}}$ and $AVD_{\text{mean, leg}}$, i.e. 9 values each per leg).

Being the parameters that quantitatively characterized blood filling changes and active vessel density, and are free of the physiological standard effect of individual animals, $PPG_{\text{ratio, area}}$, $AVD_{\text{ratio, area}}$, $PPG_{\text{ratio, leg}}$ and $AVD_{\text{ratio, leg}}$ were

introduced and calculated as

$$PPG_{ratio, leg} = \frac{PPG_{mean, leg, right}}{PPG_{mean, leg, left}},$$

$$AVD_{ratio, leg} = \frac{(AVD_{mean, leg, right} - AVD_{mean, leg, left})}{AVD_{mean, leg, left}},$$

where $PPG_{mean, leg, right/left}$ — are $PPG_{mean, leg}$ values for the right/left legs, $AVD_{mean, leg, right/left}$ are $AVD_{mean, leg}$ values for the right/left legs.

In addition, parameters calculated by one region were obtained and were written as:

$$PPG_{ratio, area} = \frac{PPG_{mean, area, right}}{PPG_{mean, area, left}},$$

$$AVD_{ratio, area} = \frac{(AVD_{mean, area, right} - AVD_{mean, area, left})}{AVD_{mean, area, left}},$$

where $PPG_{mean, area, right/left}$ are $PPG_{mean, area}$ values for the right/left legs, $AVD_{mean, area, right/left}$ are $AVD_{mean, area}$ values for the right/left legs.

The obtained data were subjected to statistical processing to determine whether there were relevant differences between the groups. The Wilcoxon criterion was used to compare $PPG_{ratio, leg}$ and $AVD_{ratio, leg}$ before and after embolization (2 groups with 10 values each for each parameter). The ANOVA method with Tukey's post hoc test was used to compare $PPG_{ratio, area}$ and $AVD_{ratio, area}$ for two legs before and after embolization (6 groups with 10 values each for each parameter), and $PPG_{mean, leg}$ and $AVD_{mean, leg}$ for two legs before and after embolization (4 groups with 60 values each for each parameter) and $PPG_{mean, area}$ and $AVD_{mean, area}$ for two legs before and after embolization (12 groups with 10 values each for each parameter). Significance of differences was determined by the criterion $p\text{-value} < 0.05$.

Results and discussion

Photoplethysmography and videocapillaroscopy results are shown in the form of box plots. Significance of differences for all described group combinations is summarized in a table. Figure 3 shows box plots of $PPG_{ratio, leg}$ and $AVD_{ratio, leg}$ (Figure 3, *a*), and $PPG_{ratio, area}$ and $AVD_{ratio, area}$ (Figure 3, *b*) before and after embolization. Data obtained before embolization on non-ischemic intact legs are marked in green. Data distributions in groups describing the differences in the intact and ischemic legs 24 hour after embolization are marked in red. Median $PPG_{ratio, leg}$ value for intact legs of animals before embolization corresponds to 1.29 arb.units. The magnitude of $PPG_{ratio, leg}$ after embolization (median value of 0.10 arb.units) which is much lower than 1 indicates a significant reduction of blood filling in the right leg tissues after embolization and ischemia in the leg. Similarly, the magnitudes of $AVD_{ratio, leg}$ after embolization (median value of -0.73 arb.units) approaching 1 in absolute value and with negative sign

indicate that the number of active vessels on skin surface is reduced, and intact legs are almost identical in this parameter (median values of -0.04 arb.units). Differences between the data groups before and after embolization appeared to be significant ($p\text{-value} = 0.0020$, table) both for $PPG_{ratio, leg}$ and $AVD_{ratio, leg}$, which confirms that the introduced parameters can be used for the quantitative evaluation of blood microcirculation during CLLI simulation.

To determine the influence of measurement location on data, $PPG_{ratio, area}$ and $AVD_{ratio, area}$ were evaluated for three test areas (hip, knee, ankle) on a leg (Figure 3, *b*). For both parameters, differences of data in the groups for three areas within one day (before and after embolization) are of a random nature and insignificant (table). In addition, for PPG , all groups before embolization have significant differences from all groups after embolization, which makes it possible to study any part of a leg. For AVD significant differences in groups before embolization were only from a group of data for the ankle. i.e. to evaluate CLLI by the vessel density, it is preferable to have vessel maps in the ankle area.

For more detailed study of blood microcirculation parameter changes in various legs and leg areas during CLLI simulation, mean standard deviations of the PPG amplitude and active vessel density were evaluated for the specified groups (Figure 4). The obtained data can be helpful for evaluating whether it is necessary to use the introduced parameters that require measurements on two legs in routine practice, and before and after embolization during generation of the differentiating criterion.

Median $PPG_{mean, leg}$ values for the left and right legs before embolization were 0.20 and 0.28 arb.units, respectively (Figure 4, *a*, table). These groups had insignificant differences between them, but differed from $PPG_{mean, leg}$ after embolization both for the left leg (median value of 0.89 arb.units) and right leg (median value of 0.09 arb.units). This can indicate that there are changes not only in the ischemic, but also in the intact leg, which have opposite trends: decrease and increase in blood filling, respectively.

However, $AVD_{mean, leg}$ for both legs before embolization and for the intact leg after embolization has no significant differences (median values of 4.13, 4.10 and 4.63 arb.units, respectively, table). $AVD_{mean, leg}$ for the ischemic leg 24 hours after the operation (median value of 1.29 arb.units) differs significantly (table) from the specified groups. Differences in the behavior of two parameters can be explained by a different depth of the test vessels: photoplethysmography can evaluate not only the state of surface vessels, but also considers the influence of arteriolas and venules on the detected signal, which agrees with [40]. While the vessel maps only reflect morphological characteristics of the capillary network (Figure 5).

Analysis of the mean standard deviation of the PPG amplitude $PPG_{mean, area}$ has shown that femoral blood filling in the intact leg after embolization has insignificant difference from values in all areas of both legs before

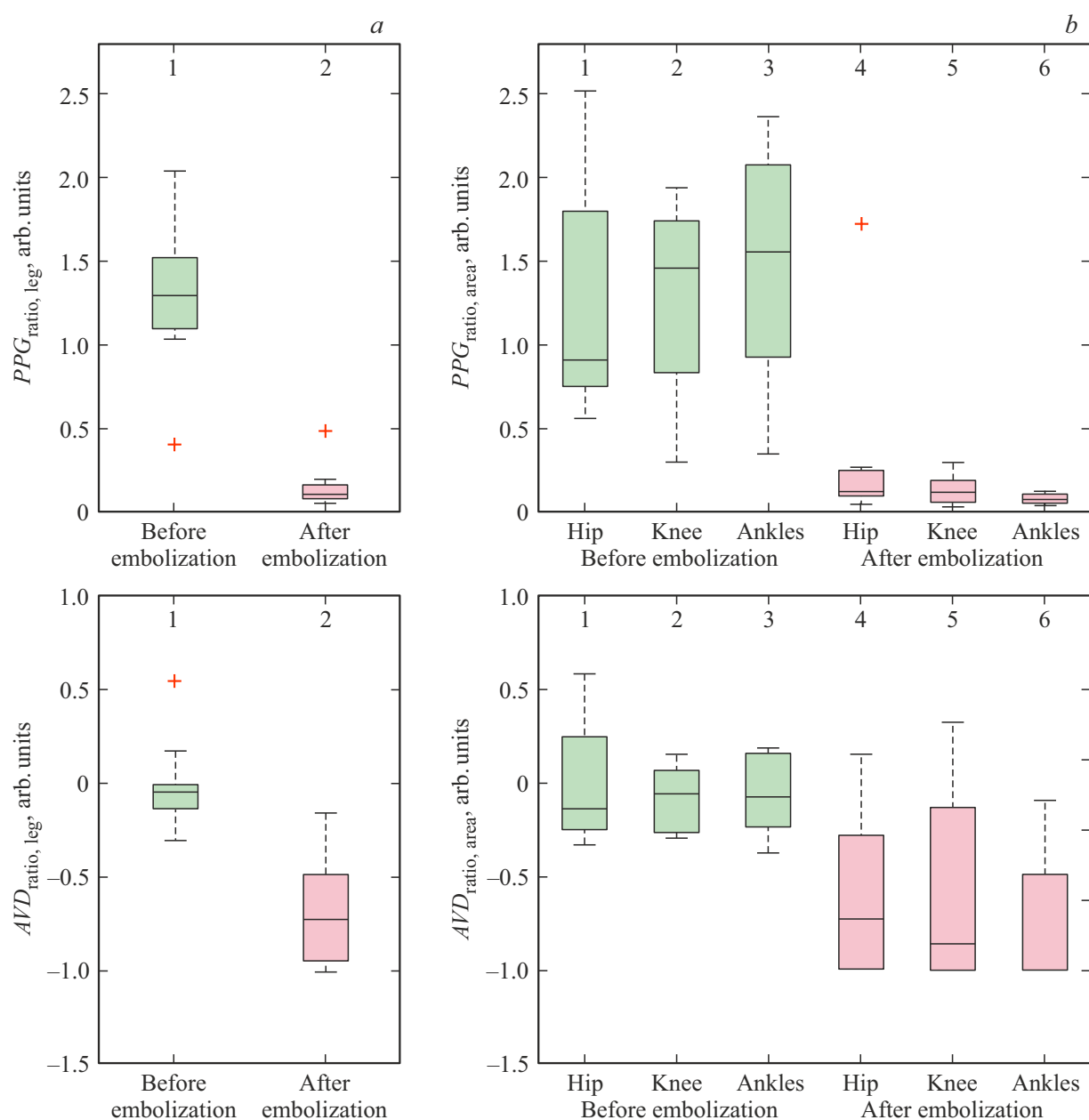


Figure 3. Diagrams characterizing the differences of blood microcirculation parameters for two legs $PPG_{ratio, leg}$ and $AVD_{ratio, leg}$ (a) and for areas $PPG_{ratio, area}$ and $AVD_{ratio, area}$ (b) before (green bars) and after (red bars) embolization. Red mark indicates outliers. A group number during the pairwise comparison using Tukey's test is shown on top.

embolization (Figure 4, b, table). This means that the knee and ankle areas make the main contribution to the increase in $PPG_{mean, leg}$ after embolization, i.e. changes are heavier for the leg end. $AVD_{mean, area}$ for the hip showed a similar absence of a significant difference from groups corresponding to the intact leg. Vessel maps for this area more often showed vascular structures for the ischemic leg, while there were almost no such structures in other areas. Such effect can be explained by the proximity of intact tissues with normal perfusion that provide angiogenesis in the hip area (Figure 5).

The obtained data suggest that, firstly, physiological features of some animals insignificantly affect the photoplethysmography and videocapillaroscopy data. Therefore, both normalized $PPG_{ratio, leg}$ and $AVD_{ratio, leg}$ and non-normalized $PPG_{mean, leg}$ and $AVD_{mean, leg}$ can be used for evaluation of the blood microcirculation state. Note that when the latter are used, no examination is required before and after embolization, which reduces the analysis time and effort. Secondly, the animal's ankle is the preferable test area, as it provides stable differentiation of the intact and ischemic legs.

Evaluation of the significance of differences: PPG standard deviation (PPG) and vessel density (AVD); (*) shows that $p\text{-value} < 0.05$ is met

Group number (as shown in the figures)		$p\text{-value}$ when comparing the data groups by various parameters and whether the significance criterion is met			
		PPG		AVD	
N^a	N^a	$p\text{-value}$	Is the criterion met	$p\text{-value}$	Is the criterion met?
$PPG_{\text{ratio, leg}}$ and $AVD_{\text{ratio, leg}}$ (number as shown in Figure 3, a)					
1	2	0.0020	*	0.0020	*
$PPG_{\text{ratio, area}}$ and $AVD_{\text{ratio, area}}$ (number as shown in Figure 3, b)					
1	2	0.9990	—	0.9999	—
1	3	0.8568	—	0.9999	—
1	4	0.0020	*	0.2378	—
1	5	0.0002	*	0.0832	—
1	6	0.0001	*	0.0103	*
2	3	0.9705	—	1.0000	—
2	4	0.0006	*	0.1508	—
2	5	0.0000	*	0.0473	*
2	6	0.0000	*	0.0051	*
3	4	0.0000	*	0.1594	—
3	5	0.0000	*	0.0506	—
3	6	0.0000	*	0.0056	*
4	5	0.9730	—	0.9958	—
4	6	0.9296	—	0.7830	—
5	6	1.0000	—	0.9669	—
$PPG_{\text{mean, leg}}$ and $AVD_{\text{mean, leg}}$ (number as shown in Figure 4, a)					
1	2	0.9152	—	0.9743	—
1	3	0.0000	*	0.7587	—
1	4	0.1444	—	0.0001	*
2	3	0.0000	*	0.5029	—
2	4	0.0352	*	0.0002	*
3	4	0.0000	*	0.0000	*
$PPG_{\text{mean, area}}$ and $AVD_{\text{mean, area}}$ (number as shown in Figure 4, b)					
1	2	0.9999	—	1.0000	—
1	3	0.9999	—	0.9994	—
1	4	1.0000	*	1.0000	—
1	5	1.0000	*	1.0000	—
1	6	1.0000	*	1.0000	—
1	7	0.3158	—	0.7962	—
1	8	0.0000	*	0.9919	—
1	9	0.0000	*	0.9997	—
1	10	0.4099	*	0.1607	—
1	11	0.4405	*	0.0472	*
1	12	0.2596	*	0.0013	*
2	3	1.0000	*	1.0000	—
2	4	0.9998	—	0.9996	—
2	5	1.0000	—	1.0000	—
2	6	0.9968	—	1.0000	—
2	7	0.0666	—	0.9834	—
2	8	0.0000	*	1.0000	—
2	9	0.0000	*	1.0000	—
2	10	0.8503	—	0.0344	*
2	11	0.8714	—	0.0075	*
2	12	0.7027	—	0.0001	*
3	4	0.9997	—	0.9950	—

Table (Continue).

N ^a	N ^a	<i>p-value</i>	Is the criterion met	<i>p-value</i>	Is the criterion met?
<i>PPG</i> _{mean, area} and <i>AVD</i> _{can, area} (number as shown in Figure 4, <i>b</i>)					
3	5	0.9999	—	1.0000	—
3	6	0.9949	—	1.0000	—
3	7	0.0567	—	0.9978	—
3	8	0.0000	*	1.0000	—
3	9	0.0000	*	1.0000	—
3	10	0.8772	—	0.0151	*
3	11	0.8959	—	0.0030	*
3	12	0.7407	—	0.0000	*
4	5	1.0000	—	1.0000	—
4	6	1.0000	—	1.0000	—
4	7	0.3533	—	0.6488	—
4	8	0.0000	*	0.9660	—
4	9	0.0000	*	0.9965	—
4	10	0.3696	—	0.2632	—
4	11	0.3989	—	0.0887	—
4	12	0.2286	—	0.0030	*
5	6	1.0000	—	1.0000	—
5	7	0.2922	—	0.9383	—
5	8	0.0000	*	0.9996	—
5	9	0.0000	*	1.0000	—
5	10	0.4377	—	0.0694	—
5	11	0.4689	—	0.0171	*
5	12	0.2816	—	0.0003	*
6	7	0.5429	—	0.9341	—
6	8	0.0000	*	0.9996	—
6	9	0.0000	*	1.0000	—
6	10	0.2157	—	0.0723	—
6	11	0.2372	—	0.0179	*
6	12	0.1208	—	0.0004	*
7	8	0.0120	*	0.9999	—
7	9	0.0034	*	0.9967	—
7	10	0.0002	*	0.0004	*
7	11	0.0002	*	0.0001	*
7	12	0.0001	*	0.0000	*
8	9	1.0000	—	1.0000	—
8	10	0.0000	*	0.0056	*
8	11	0.0000	*	0.0010	*
8	12	0.0000	*	0.0000	*
9	10	0.0000	*	0.0174	*
9	11	0.0000	*	0.0035	*
9	12	0.0000	*	0.0000	*
10	11	1.0000	—	1.0000	—
10	12	1.0000	—	0.9224	—
11	12	1.0000	—	0.9951	—

Data obtained using the photoplethysmography and videocapillaroscopy were supported by the MR angiography which showed that arterial perfusion in the femoral artery was terminated below the embolization zone. Arrow in Figure 6 shows an area below which the arterial perfusion is blocked (marked in red). Microcirculation disorder in the ischemic leg zone after embolization was also confirmed using the MR perfusion after bolus injection of the MR contrast (Gadovist) (red and yellow polygons in Figure 7, *a*

show soft tissue areas on the intact and ischemic legs, respectively). It is evident that perfusion redistribution in the affected leg — a curve in Figure 7, *b*, shows that perfusion in the ischemic leg is significantly reduced compared with the intact one.

Thus, the data obtained in this study via optical methods agree with the data from the MR angiography and MR perfusion that are traditional for the given task. Moreover, the proposed optical methods additionally contain information

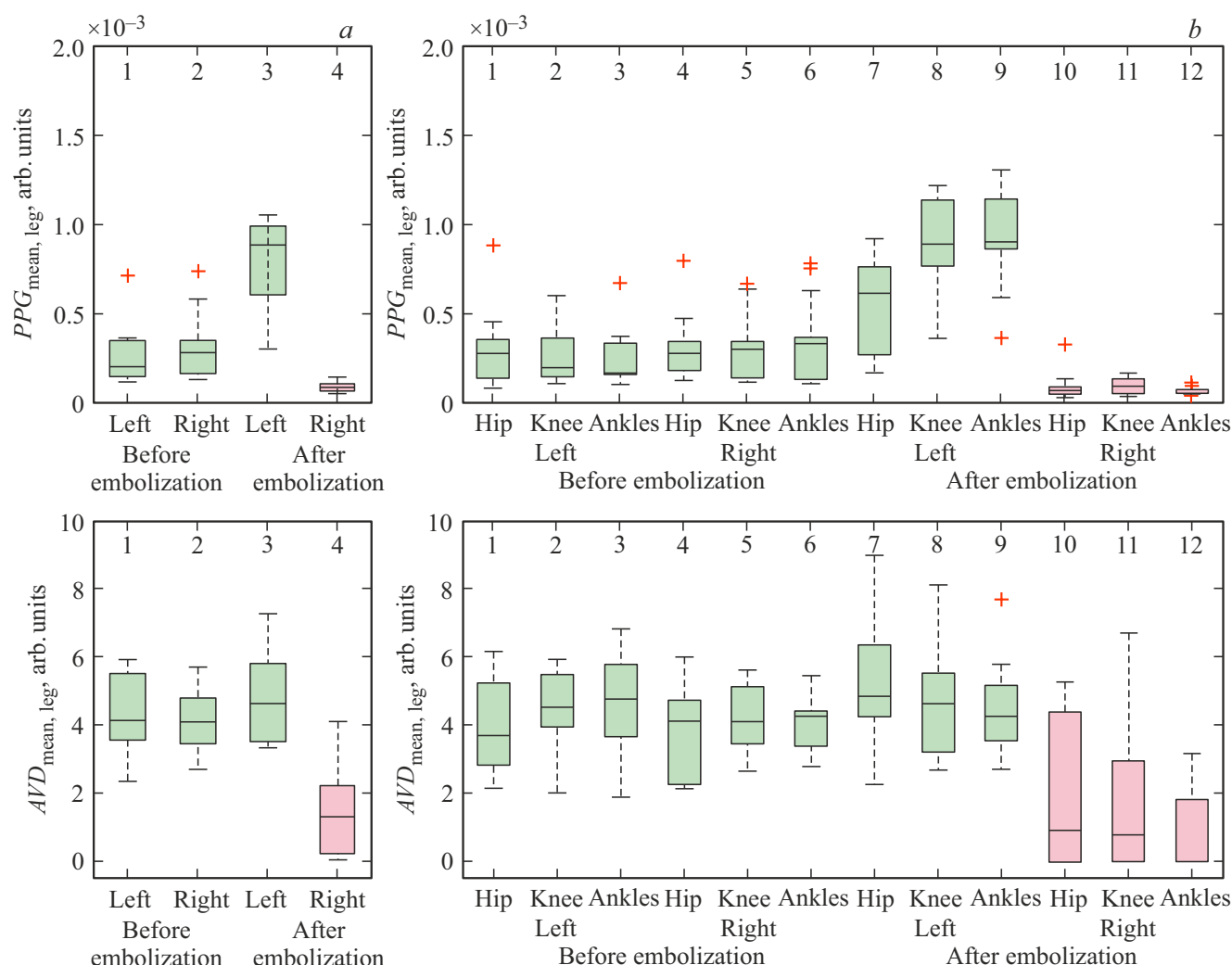


Figure 4. Box plots of blood microcirculation parameters for two legs $PPG_{mean,leg}$ and $AVD_{mean,leg}$ (a) and leg areas $PPG_{ratio,area}$ and $AVD_{ratio,area}$ (b) before and after embolization. Data groups for intact legs are marked in green, for ischemic legs are marked in red. Red mark indicates outliers. A group number during the pairwise comparison using Tukey's test is shown on top.

about the surface microcirculation in the region of interest. They are noninvasive and differ from the MR perfusion and some other optical methods in simplicity of engineering implementation, lower requirements for animal preparation and high analysis speed.

Note that the developed approach has some constraints: relatively high data nonhomogeneity for various skin zones and requirement to perform more than one measurement to improve the analysis reliability. Moreover, soft tissue volume available for analysis is limited to a depth of about 0.5 mm (for a wavelength of 532 nm) and a field of view of 2.5×2.5 mm (with $2\times$ magnification of the optical system and a matrix receiver diagonal of $1/1.8''$).

Conclusion

Possibility of using photoplethysmography and videocapillaroscopy for experimental CLLI monitoring has been

investigated. CLLI simulation and evaluation of CLLI-induced changes in blood microcirculation parameters have been performed using the above-mentioned techniques. It is shown that they can reliably measure parameters demonstrating significant differences in the ischemic and intact legs. They include the standard deviation of the PPG amplitude describing a blood filling change with time, and a vessel map that provides imaging and quantitative evaluation of the spatial distribution of the vessel network in the region of interest. It is shown that both normalized and non-normalized parameters, i.e. parameters that account and don't account for the physiological variability, respectively, have a similar stability. Ankle has been chosen as a preferable test area in terms of reliability of results. Individual features of changes in the microcirculation state after embolization have been identified, including an increase and decrease in blood filling of the intact and ischemic legs, respectively, and maintenance at the initial level and

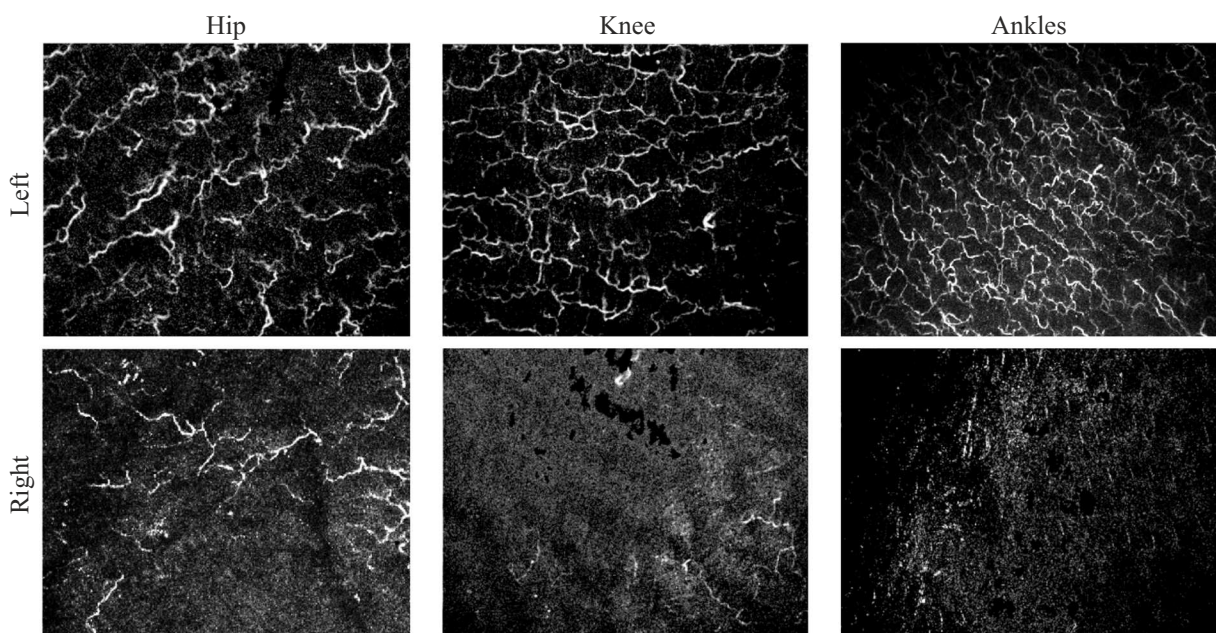


Figure 5. Vessel maps obtained in different areas of the intact leg (upper row) and ischemic leg (lower row) 24 hours after embolization.

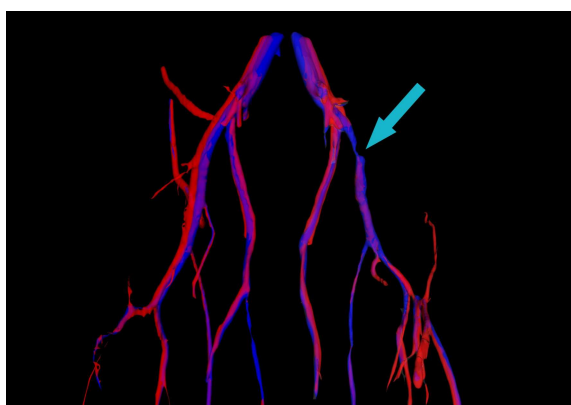


Figure 6. Example of an image made by the MR angiography of the ischemic leg. 3D reconstruction of leg vessels: arteries are shown in red, veins are shown in blue. MR angiography was performed using the ClinScan 7T scanner (Bruker).

reduction of the active vessel density in them. It is shown that femoral blood microcirculation is less susceptible to ischemia in the given protocol.

Results obtained using the proposed techniques agree with the MR angiography and MR perfusion data that are traditional examination techniques for the given task and supplement the MR angiography and MR perfusion in terms of surface microvessel analysis. The proposed approach is noninvasive and almost has no effect on the animal health, and therefore can be used in tests for long-term routine monitoring. Thus, photoplethysmography and videocapillaroscopy can be used to expand the diagnosis and health evaluation capabilities.

These methods are relatively simple in terms of hardware and software implementation. Equipment for their implementation is compact and can be successfully built in the existing laboratory systems, and data acquisition and processing don't require any complex preparation and can be performed quickly enough — examination of one animal within the experiment took up to 10 min. Therefore, the proposed approach can be a noninvasive, informative, quantitative, reliable and, thus, important supplement to the existing methods for perfusion evaluation in soft tissues for diagnosis and evaluation of vascular diseases.

Compliance with ethical standards

The test protocol was approved by the Local Ethics Committee of the Federal Scientific and Clinical Center of the Federal Medical and Biological Agency of Russia (minutes of the Local Ethics Committee meeting № 1.1.2025 as of 11.02.2025).

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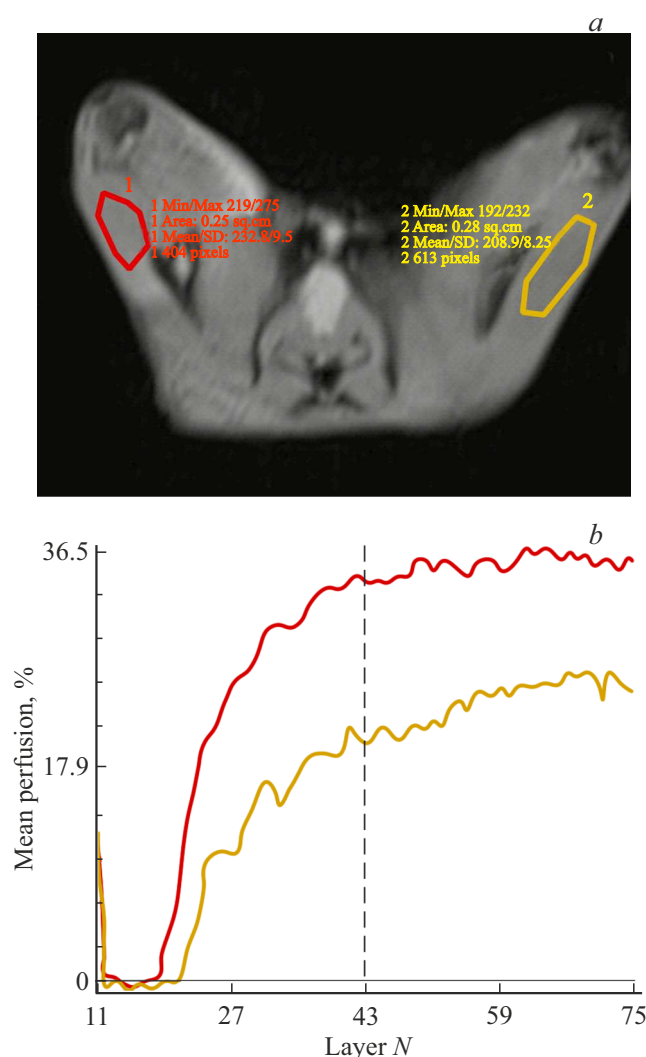


Figure 7. MR perfusion of legs: (a) regions of interest corresponding to the intact (red) and ischemic (yellow) legs; (b) perfusion in each section averaged over two regions of interest. The study was performed immediately after intravenous administration of the MR contrast (0.05 ml Gadovist solution) using the ClinScan 7T scanner (Bruker).

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Declaration of competing interest

The authors declare no conflict of interest.

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