Stimulation of Blood Microcirculation at Low Level Laser Therapy: Monitoring Tools and Preliminary Data

D.A. Rogatkin[1], A.V. Dunaev[2]

[1]Laboratory of Medical and Physics Research of Moscow Regional Research and Clinical Institute "MONIKI" named after M.F. Vladimirskiy, Shepkina str. 61/2, Moscow, 129110, Russia

[2]Scientific-Educational Center "Biomedical engineering" State University – Educational-Scientific-Production Complex, Naugorskoe shosse 29, Oryol, 302020, Russia

[1]rogatkin@monikiweb.ru; [2]dunaev@ostu.ru

***Abstract*- The paper discusses the possibilities of noninvasive optical spectrophotometric diagnostic techniques for registration and evaluation of changes in blood microcirculation processes at low level laser therapy. Preliminary results of experimental studies are presented. Visible stimulation of blood microcirculation was steadily observed only in the case of tissue heating on 0.8...1 ^{0}C or more.**

Keywords - Low Level Laser Therapy; Microcirculation of Blood; Laser Doppler Flowmetry; Tissue Reflectance Oximetry; Thermography

I. INTRODUCTION

In the modern medicine, especially in Russia as well as in a number of other East-European countries, different physiotherapeutic methods of treatment and prevention of diseases are widely used. These methods cover application of various natural or artificially created healing physical factors of mechanical, thermal or electromagnetic nature. In the past 20-25 years special area among them was occupied by low level laser radiation (LLLR). Therefore, in 1980-2000 besides a laser surgery the intensive evolution of low level laser therapy (LLLT) had started in medicine, especially in Russian physiotherapy [1]. At the same time, in serious literature, especially in developed European countries, USA, Japan, etc. biophysical mechanisms and a real clinical effect of LLLT are still a subject of disputes. Today one may speak of at least 4-5 existing initial mechanisms of LLLT: thermal effect (heating of tissue), photodynamic one (activation of singlet oxygen), photochemical effect (direct photodestruction of organic molecules), placebo effect and so on. Meanwhile the healing effect of LLLT is far not always reproducible or guaranteed. There is in the literature a lot of inconsistent information concerning LLLT. Take just speculations on dosage determination in LLLT! Recommended settings for the useful power density in the different guidelines differ in hundred times or more (from 0.5 up to 200 mW/cm^2), and the recommened "dose" (energy density) ranged from 0.1 up to 120 J/cm^2 [2]. Even the World Association for Laser Therapy (WALT) had formulated some recommendations on effective doses for LLLT by 2010 only, and only for wavelengths of 780-860 nm (continuous or pulsed mode) and 904 nm (pulse mode). But the recommended doses are still in a very wide range, 1…6 J per area (six times!) of irradiation at a power density of more than 5 mW/cm^2 [3]. Thus, the problem is opened yet.

In our opinion, one of the reasons of such situation in LLLT is an insufficient application of objective methods for direct and quantitative registration and visualization of the direct therapeutic effect during each LLLT procedure on both a local and a system level (blood microcirculation system, cell metabolism, etc.) as well as on a total level of the organism (control of basic parameters of homeostasis). For example, a stimulation of the blood microcirculation is described in most Russian medical articles as one of the basic responses on LLLR or one of the basic positive biological effects of LLLT [4, 5]. And most authors are confident in this case that no substantial heating of the tissues occurs at the LLLT procedures, i.e. that the temperature of the irradiated tissues does not increase by more than 0.1^{0}C [4, 6, 7]. These conclusions as a whole were obtained about 10–15 years ago on the basis of ordinary clinical observations and the results of laboratory and morphological studies. There were no devices available to physicians at that time to record microcirculation processes and the dynamics of the surface temperature of biological tissues with sufficient accuracy and in real time. Today such devices have appeared. These include, most importantly, noninvasive spectrometric instruments that make it possible to monitor tissue respiration and the perfusion of tissues with blood [8] as well as apparatus for digital infrared (IR) thermography and thermal-vision monitoring devices that record the temperature over a large surface area of the body within $\pm$0.05^{0}C [9]. All this opens up the prospect of direct and on-line checking of conclusions made earlier and some of the most controversial and interesting results of earlier papers (as is well-known, only results that are independently verified in different laboratories of the world can be regarded as reliable in the science).

In this paper we would like to offer for discussion our preliminary results on studying of the reaction of the blood microcirculation system on LLLR, executed at LLLT in the conditions of monitoring of blood microcirculation parameters and of a superficial tissues' temperature by modern noninvasive spectrophotometric methods and apparatus. It should be specially noted

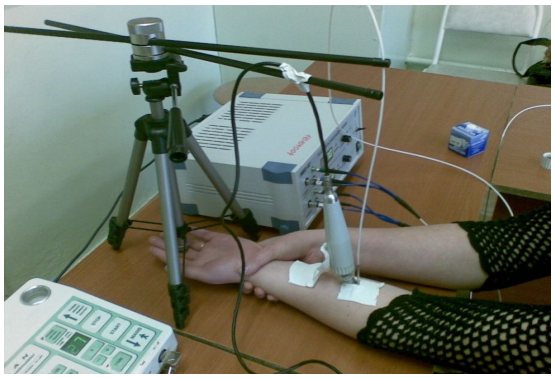
that the initial attempts of application of the noninvasive medical spectrophotometry (NMS) techniques (Laser Doppler Flowmetry (LDF) or Reflectance Tissues Oximetry (TRO)) to assess the response of the blood microcirculation system on LLLR have taken place [10, 11], but in general they were not systemic and did not combine measurements of the tissues' temperature together with the spectrophotometric measurements of microhaemodynamic parameters.

II. MATERIALS AND METHODS

To register parameters of blood microcirculation we used the multifunctional noninvasive laser diagnostic system "LAKK-M" (SPE "LAZMA", Russia), which provides one-time measurement of tissue blood perfusion (index of blood microcirculation I_m in the perfusional unit (PU)), oxyhemoglobin saturation in the mixed peripheral blood (tissue's oxygen saturation - S_tO_2) and relative erythrocyte (red blood cells) fraction volume (volume of blood - V_b) in the probed region of tissues [8]. It became possible thanks to association of two different diagnostic methods - LDF and TRO - in one diagnostic system. All mentioned parameters can be registered *in vivo* by the system's optical fiber probe as a function of time in a real time mode. After the registration some additional calculations, wavelet analysis, for example, are available to obtain the frequency variations (δ) of all registered parameters. It is well-known that the frequency rhythms in the microhaemodynamics reflect different physiological regulation mechanisms for the microcirculatory vascular bed, so the analysis of any changes in the registered blood flow rhythms from the microcirculation system both at LLLT procedures and after them can reflect the response of the organism on the LLLR as well [5, 8].

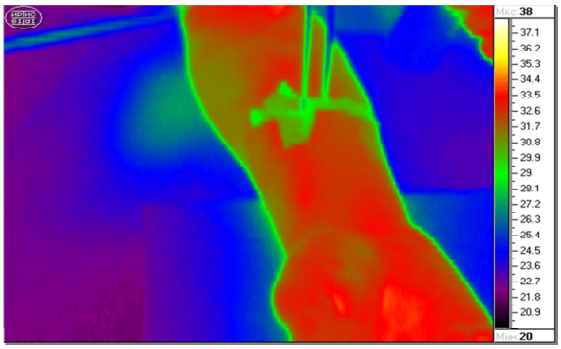
To measure the temperature field on a surface of irradiated tissue we used the medical thermograph "IRTIS ME-2000" [7] (Institute of Radio Engineering and Electronics, Russian Academy of Sciences), which has an absolute error of measurement ±0.05°C and a sensitivity of 0.05°C as well. Systems "LAKK-M" and "IRTIS ME-2000" were always used together to find out a correlation between temperature changes and the blood microcirculation ones. For studying in comparative aspect the changes in blood flow at the conventional heating test the standard block "LAKK-TEST" (SPE "LAZMA", Russia) with a manual heating probe and a thermostabilization system (40...42°C) was used. To carry out the LLLT procedures we used both the laser therapeutic device "ULAN-BL-20" (λ=0.89 μm, the pulse mode up to 30 kHz) and the physiotherapy laser system "ULF-1" (λ=0.632 μm, continuous mode, the power of 20 mW).

All experiments were performed with the help of 10 healthy volunteers (authors of the paper in that number). Some part of LLLT procedures was carried out with the use of continuous mode of irradiation and another one with the use of a pulsed mode of LLLR influence. The irradiated area – skin of the palmary surface of hands, skin of a reverse side of the hand, skin of foots and of head and neck. Photographs of the experimental setup and the fragments of the conditions of the research are presented in Fig. 1. Thermograph camera was set so that it was possible to measure the temperature in the area of impact.



a)

b)



c)

Fig. 1 Experimental design for irradiating hand's skin by laser. Irradiating by "ULAN-BL-20" (a), the visible irradiation λ=0,632 μm by "ULF-1" and diagnostic fiber probes (b), the same like (b) in the thermal IR image (c) with the temperature scale.

A total number of experiments were divided into 3 sets. The first set was carried out with the use of real laser irradiation (LLLT procedures). The second one was carried out like a placebo experiments (no impact) – without real irradiation of the skin. And in the third set of experiments we used a conventional heating test (contact heating) to register a dynamics in blood microcirculation for the comparison. Also, the first set of experiments was divided into 2 groups: the first group with the procedure of LLLT with the use of laser device "ULAN-BL-20" and the second group with the procedure of LLLT with the use of the laser system "ULF-1". To be able to assess correctly the dynamics of blood microcirculation all measurements were performed before, during and after LLLR exposure with a total time of each experiment 8-10 min and a time of exposure of around 5-6 min. Temperature tests (TT) as well as all "placebo" experiments had the same temporal parameters within 8 min of a total time of records. Additionally, after the first results of the experiments were analyzed a number of placebo records were repeated with the double time up to 16 min of registration.

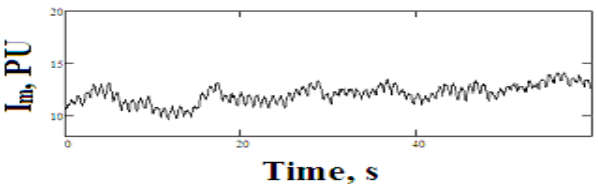
At the data processing for all experiments, all groups of recorded signals were divided into three fragments: "0" – a fragment, prior to being exposed and control, "B" – a fragment corresponding to the effects of LLLT procedure and the heating time for TT, and "K" – a fragment corresponding to the time after exposure. LDF signal during the procedure LLLT with the use of the pulse mode of LLLR (device "ULAN-BL-20") was not analyzed due to the direct influence of the pulsed IR laser light on the LDF registration channel of the "LAKK-M" system.

To evaluate results of experiments for each registered blood microcirculation parameter an arithmetic mean value ($\overline{Q}_0$, $\overline{Q}_B$, $\overline{Q}_K$) as well as its standard deviations (S_{Q0}, S_{QB}, S_{QK}) was calculated for each fragment "0", "B" and "K" (respectively, before, during and after exposure). Wavelet analysis was used for the registered parameter I_m to visualize the contribution of different regulatory rhythms into the frequency variations of the registered blood flow (δI_m).

III. RESULTS AND DISCUSSION

Examples of the brightest fragments of changes of I_m parameter (LDF-grams) as well as their output of wavelet analysis before and after pulse LLLR exposure (fragments of "0" and "K") are shown in Fig. 2. Parameters of the LLLT procedure were in this case as follows: pulse power – 7 W, frequency – 30 kHz, pulse duration – 200 ns, the area of exposure – 2 cm^2, energy exposure – 6,3 J/cm^2. On these schedules it is possible to see distinctly visible increase in a blood flow (in I_m) from around 12…14 PU (in average) up to 15…18 PU (increase of around 25…30% from the initial level) and appreciable increase in a contribution of neurogenic and miogenic mechanisms in the blood microcirculation regulation. For the purpose of more numerical assessing of changes in the rhythms of blood microcirculation the ratio of standard deviations (S) of the LDF signals before and after the treatment effects can be calculated. For the example in Fig. 2 this ratio was 2.5, indicating a significant increase in the oscillation amplitudes of the rhythms of blood microcirculation after the LLLT procedure. However, we have to say that such picture was found out in one of our experiments only. All other experiments showed essentially smaller changes in all parameters of microhaemodynamics (see below).

The most typical dependences of the surface temperature of biological tissues for each group of experiments including "placebo" and "TT" are shown in Fig. 3. The graphs also assessed average temperature in 3 fragments (intervals) of observations (dotted line – the beginning and the end of the LLLT/TT procedure).



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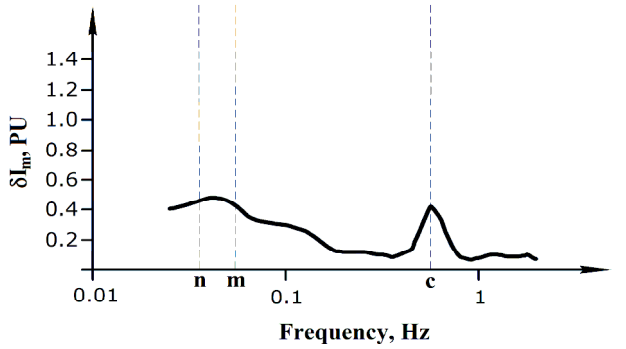

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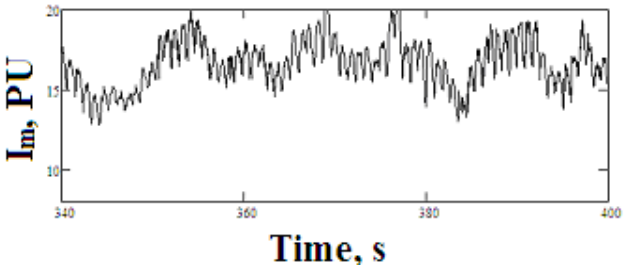

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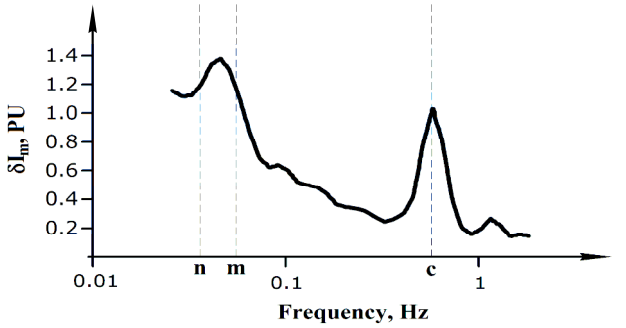
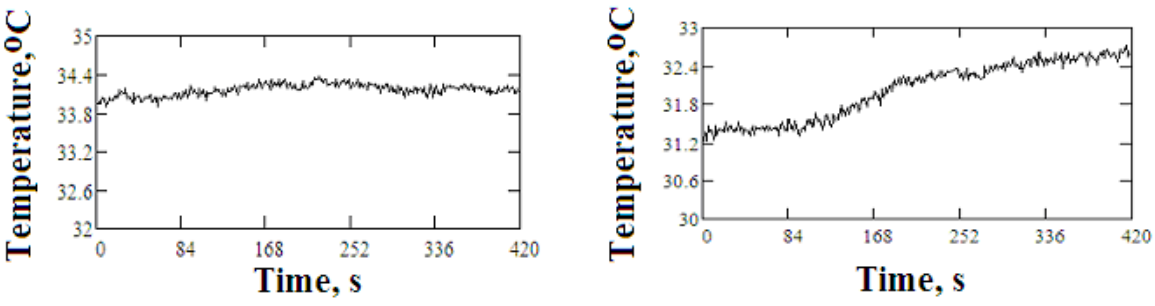


d)

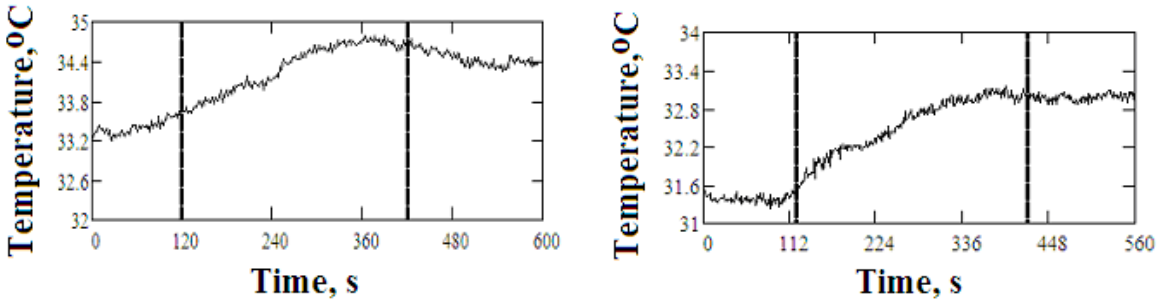
Fig. 2 Examples of typical fragments of LDF-grams (a, c) and their wavelet analysis (b, d) before (a, b) and after (c, d) LLLT procedure: n – neirogen rhythms; м – miogens rhythms; c – cardiorhythms

For the LLLT procedure (d) it is shown in Fig. 3 once more our more visible results under CW mode irradiation at a power density of about 50 mW/cm^2 on the skin of dorsal side of the radiocarpal joint for one of our volunteers. In this case the temperature increase was around 1.2…1.3^{0}C together with some synchronous and correlated increase of all other parameters of blood microcirculation (I_m, S_tO_2 and V_b). The same was observed at all TT tests (Fig. 3c). The temperature increase on 1…2^{0}C in a tested zone caused steady increasing in parameters of microcirculation that was rather obvious. Without any impact on the skin the registered fluctuations of superficial temperature were in limits of 0.8…1^{0}C, and they did not correlate generally with physiological fluctuations in blood microcirculation parameters any more.

For analysis some fragments of our total results on three experiments in each set are presented in Table 1.



a) b)



c) d)

Fig. 3 Examples of dependence of tissue's surface temperature versus the time of the experiment.
(a, b) - "placebo" group, (c) - TT, (d) - LLLT.

TABLE 1 EXAMPLES OF THE EXPERIMENTAL RESULTS

Parameter	Estimation of parameters in the fragments	Name of experiments											
		No impact			LLLT (ULAN)			LLLT (ULF)			Temperature test		
		№ of experiment											
		1	2	3	4	5	6	7	8	9	10	11	12
I_m, PU	$\overline{Q_0}$	5,03	5,41	7,94	9,29	8,85	11,91	4,28	2,70	9,49	7,16	2,95	2,20
	S_{Q0}	0,44	0,67	0,94	0,47	0,86	0,75	0,44	0,44	0,53	0,46	0,14	0,27
	$\overline{Q_B}$	5,28	6,28	7,70	-	-	-	5,54	2,65	8,77	8,29	3,24	3,07
	S_{QB}	0,57	1,18	1,52	-	-	-	0,37	0,41	0,99	0,92	0,14	0,20
	$\overline{Q_K}$	4,94	6,81	7,29	8,60	8,62	16,88	4,91	3,90	9,42	10,30	4,25	3,53
	S_{QK}	0,49	1,09	1,54	0,56	0,91	1,88	1,00	0,77	1,11	0,87	0,19	0,33
S_tO_2, %	$\overline{Q_0}$	79,06	67,58	51,25	50,50	62,92	34,98	69,30	81,93	48,52	55,00	40,35	45,94
	S_{Q0}	0,36	1,33	1,67	0,66	0,75	0,75	1,23	1,19	0,64	0,25	0,18	0,33
	$\overline{Q_B}$	78,18	67,56	52,61	49,68	60,70	34,36	71,76	84,10	48,41	55,59	40,76	45,78
	S_{QB}	0,23	1,46	1,72	0,84	0,70	0,74	0,44	0,26	0,56	0,16	0,14	0,17
	$\overline{Q_K}$	78,01	68,47	53,46	46,36	58,13	39,78	68,95	82,56	49,04	56,40	37,58	44,24
	S_{QK}	0,26	1,27	2,02	0,70	0,69	1,17	0,57	0,31	0,80	0,24	0,15	0,34
V_b, %	$\overline{Q_0}$	14,71	10,83	10,22	14,21	13,27	8,18	11,12	9,07	13,77	10,35	10,67	12,41
	S_{Q0}	0,17	0,20	0,36	0,14	0,26	0,07	0,24	0,58	0,15	0,05	0,15	0,15
	$\overline{Q_B}$	14,29	11,29	10,63	14,47	12,46	8,10	11,75	10,09	13,84	10,88	11,27	12,98
	S_{QB}	0,11	0,38	0,30	0,10	0,22	0,12	0,13	0,10	0,11	0,04	0,06	0,06
	$\overline{Q_K}$	14,33	11,65	11,13	14,52	11,68	9,42	11,49	10,60	14,31	11,86	12,87	13,94
	S_{QK}	0,17	0,30	0,44	0,12	0,16	0,20	0,24	0,17	0,17	0,05	0,11	0,13
T, °C	T_0	34,06	31,43	32,48	33,46	33,93	33,34	30,11	31,12	34,33	31,54	32,77	33,39
	T_B	34,24	32,00	32,06	33,84	34,74	-	30,00	31,51	34,69	32,38	33,39	34,36
	T_K	34,19	32,42	32,11	34,47	34,50	-	31,91	31,71	34,44	33,87	34,53	34,48

The results of our studies on the whole thus confirmed the latest other experimental results of Refs. [7, 10-12], that variations of the parameters of the microcirculation and oxygenation of the blood at LLLT occur much less often and are less clearly expressed (if they are there at all) than reported by Russian purely medical primary sources. All *in vivo* measured parameters of blood microcirculation as well as of superficial temperature have natural physiological uncorrelated fluctuations

and drift in the limit of about 10...12% from the average parameter's values during the observation period in the absence of any impact. So, under light irradiation we always see the same uncorrelated fluctuations and cannot make a valid decision about induced by LLLR changes in the blood microcirculation if they are lower than this threshold and are registered by one of the used techniques only (by temperature measurements only, for example). But with the use of simultaneous measurements of a set of parameters like we have done one can make a valid decision by means of analysis of correlations in all changes. In our experiments if there were induced changes then all registered parameters behaved synchronously. And all these changes towards stimulation of a blood flow were accompanied by the corresponding growth of the skin surfaces temperatures on 0.8…1 ^{0}C or more. It was reached either at usual contact heating or at skin exposure with a power density more than 50 mW/cm^2 [7]. Below this threshold non-synchronous physiological variations of all parameters without their visible communication with any external influence were observed. So, today we have a quite proved opinion that the conventional heating is the dominant mechanism of the LLLR actions on living tissues.

A Japanese publication [13] is instructive in this sense and has existed for a long time but has apparently remained unnoticed by medical specialists. It reported the action of LLLR of different wavelengths and power densities on the vascular tone in an experiment with individual segments of vessels. For comparison, the reaction of the vessel to ordinary heating was also tracked as well. The fundamental conclusion of that article is that the vascular tone reacts only to heating, regardless of by what method it is obtained, by contact heating or by means of LLLR. In all cases of irradiation and contact heating a reaction of the vessels was observed in this experiment only when the temperature in the zone of action increased by about 1^{0}C from the initial level. Neither contact heating nor LLLR below this threshold produced an appreciable change of the vascular tone, and this is consistent with our results of the presented study. Though the physiological sense of this threshold in 0.8…1^{0}C remains yet not absolutely understood, but as a whole results of different researches in all these cases meet to a uniform conclusion.

As to the LLLT output in the form of enhanced regulatory rhythms in the system of blood microcirculation (see Fig. 2), which can be generally not necessarily correlated with the increase of the skin temperature or with other microhaemodynamic parametres, this fact demands additional researches. In our study we observed the similar phenomenon for several times, however, similar changes from time to time were fixed in placebo experiments as well. For example Fig. 4 presents a natural fluctuation in the index of microcirculation I_m (blood flow) at the absence of any action (impact) on the hand's skin in the norm. In this "placebo" test I_m was registered during 16 minutes (double time of the experiment) from the palmary surface of skin of a third finger distant phalanx while the hand motionlessly lay at the level of the examinee's heart.

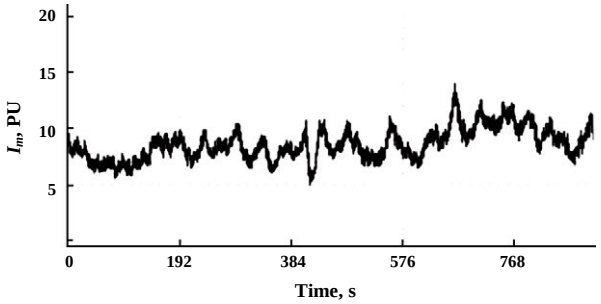


Fig. 4 Natural fluctuations of the blood flow (I_m) at the absence of any impact

On the graphics the increase in amplitudes of low-frequency fluctuations of a blood flow after 200 seconds of the record in an explicit form is visible. It can be a consequence of a small hypostasis of a hand in a motionless condition as well as of an attempt of the examinee's organism to compensate a lack of blood outflow by the increase of low-frequency vasomotions of small vessels of a microcirculatory bed. I.e. the blood microcirculation system is very variable and also is very adaptable under any external influences, so the direct interpretation of changes of its parameters is not always possible in terms of the induced changes owing to only influence of LLLR. Therefore only comparative experiment for enough big groups of examinees with influence of LLLR and without one can answer the question which prevails in these cases - changes from hypostasis in the conditions of limited mobility of an extremity, or direct effect of laser radiation.

IV. CONCLUSION

Thus, both by modern digital thermography and by combined two modern methods of NMS (LDF and TRO) the new non-evident experimental data were obtained on stimulation of the blood microcirculation directly at the LLLT procedures and right after that. We did not obtain proved confirmation that LLLT has unequivocal stimulating effect on the blood microcirculation system in skin at a power density below 50 mW/cm^2 with irradiation time of 5-6 minutes. Above this threshold the data on the most probable heating on 0.8…1^{0}C of skin in the field of irradiation and on the corresponding synchronous increase of all parameters of microhaemodynamics were obtained that are coordinated with other data of other authors. The question of possibility of the increase of low-frequency rhythms of regulation of a blood microcirculation at LLLR still remains open and demands additional researches, because the system of microcirculation is very variable and very adaptable under and without any external influences. Also additional researches are demanded by a problem of an explanation of a threshold of temperature

heating in $0.8…1^0$C.

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Dmitrii Rogatkin was born in 1962. He received the initial mechanical engineer degree in 1985 from Moscow High Technical School named after N.E. Bauman. In 1994 working as a senior researcher at the Laboratory of Laser Medicine of Moscow Regional Research and Clinical Institute "MONIKI" named after M.F.Vladimirskiy he received the Ph.D. degree from Russian State Academy of Science as well as in 2005 the degree of Doctor of Technical Sciences. Professional area of activity – medical physics and R&D of optical and electronic medical diagnostic equipment. Dmitrii Rogatkin now heads the Laboratory of Medical and Physics Research in Moscow Regional Research and Clinical Institute "MONIKI" named after M.F.Vladimirskiy. This is the first in Russia medical physics laboratory, created in 2009 in the biggest state medical research center to solve different problems of non-ionizing medical physics for the practical medicine (www.medphyslab.com). He is an author of about 200 articles in journals and about 25 Russian patents. Also he is an associate professor of Moscow State Institute of Electronics and Mathematics.

Andrey Dunaev was born in Kishinev, USSR in 1976. He received his diploma in Instrumental Engineering (MSc) and diploma in Management (MSc) at Oryol State Technical University (now – State University ESPC), Russia, in 1999. In 2002 he was awarded a PhD degree for his work "Method of control of the absorbed radiation power in epidermis during low level laser therapy". Since 2005 he was associate professor (docent) of Department "Device-making, metrology, certification" at Oryol State Technical University. He was teaching lectures on "Laser and light apparatus in physiotherapy and surgery", "Optoelectronic Devices in Diagnostics, Therapy and Surgery", "Basis of biospectrophotometry" etc. Since 2010 he is chief executive Science-Educational Center "Biomedical Engineering" at State University ESPC. He is an author and co-author of books (in Russian): "Laser therapeutic apparatus" (Russia, Oryol, OSTU, 2005, 143 p.); "Physical and technical bases of low-level laser therapy" (Lambert Academic Publishing, 2012, 286 p.). His research interests include the development of biomedical non-invasive optical diagnostics methods and devices – such as laser Doppler flowmetry, tissue reflectance oximetry, laser fluorescence diagnostic, etc. Topics of research: physical and technical aspects of low-level laser therapy, methods and means for diagnostics of the functional state of peripheral vessels, metrological support of devices for laser Doppler flowmetry.