# Evaluating adaptation options of microcirculatory-tissue systems based on the physiological link of nutritive blood flow and redox ratio

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## ABSTRACT

Fluorescent spectroscopy (FS) is becoming more widely used in chemistry, biology, in various fields of medical technology and medicine in general. Many purulent wounds, burns and other destructive inflammatory processes are accompanied by changes in the fluorescent activity of the tissues, which occurs due to a misbalance in accumulation of natural fluorophores: FAD, NADH, lipofuscin, porphyrins, structural proteins, etc. The study of redox ratio (RR), characterizing the metabolic processes, is important in the assessment of the metabolic activity ofmicrocirculatory-tissue systems (MTS). However, one of the big problems of the FS method is still the correct interpretation of the data and the development of practical methods for its application in clinical medicine.

To solve this problem and create new diagnostic criteria, we propose to evaluate the adaptive capacity of MTS using indicators of links between nutritive blood flow and redox ratio during a physiological rest and functional load (occlusion test). As is known, these parameters (RR and nutritive blood flow) characterize the metabolic activity of tissues.We have performedan experimental study of the relationship between the RR, defined by FS, and nutritive blood flow, defined by the methods of laser Doppler flowmetry.

Preliminary results in the study of a complex approach to diagnosis of the state of biological tissue were obtained. A positive relationship between the nutritive blood flow in the microcirculatory channel and RR of skin tissue is observed.The speed of change of metabolism in the phase of occlusion and reperfusion and duration of phase of recovery may be the criteria for adaptive capabilities of MTS, which has practical significance for physiology and medicine.

**Keywords:** fluorescence spectroscopy, redox ratio, metabolism, laser Doppler flowmetry, nutritive blood flow, microcirculatory-tissue system

## 1. INTRODUCTION

Quantitative spectroscopic analysis methods, such as fluorescence spectroscopy (FS), are a rapidly developing area of medical optical diagnostics[1, 2]. These methods are highly sensitive and provide a unique opportunity to study the excited states of molecules, photochemical reactions, dynamics of fast molecular processes, structures, and properties of complex biochemical and cellular systems. The FS method is based on exciting fluorescence from tissue endogenous and exogenous fluorophores and recording the emission in the visible spectral region.The FS provides effective and non-invasive optical diagnostics, primarily in medical areas such as oncology, transplantation, cosmetology and surgery[3-5].

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Saratov Fall Meeting 2014: Optical Technologies in Biophysics and Medicine XVI; Laser Physics and Photonics XVI; and Computational Biophysics, E. A. Genina, V. L. Derbov, K. V. Larin, D. E. Postnov, V. V. Tuchin, Eds., Proc. of SPIE Vol. 9448, 944803 · © 2015 SPIE · CCC code: 1605-7422/15/$18 · doi: 10.1117/12.2179560

The delivery/consumption law is a key part of maintaining a balance of function of microcirculatory-tissue systems (MTS). In particular, the study of redox-processes (redox ratio – RR) is important in the assessment of the metabolic activity of tissues[6]. However, in general, one of the problems of the fluorescence spectroscopy method is still the correct interpretation of the data and the development of practical methods for its application in clinical medicine.

The aim of this work was evaluating the adaptive capacity of MTS using indicators of links between nutritive blood flow and redox ratio during a physiological rest and functional load (occlusion test) states. For this purpose, an experimental study of the relationship between the RR (defined by FS) and nutritive blood flow (defined by the methods of laser Doppler flowmetry (LDF)) were performed. As is well known, these parameters (RR and nutritive blood flow) characterize the metabolic activity of tissues and with active metabolism should see an increase in the redox ratio of NADH/FAD. When analysing the relation of nutritive blood flow and redox ratio during the occlusion test, we turned to the so-called metabolic theory of blood flow regulation[7, 8].

## 2. MATERIAL AND METHODS

Experimental research was conducted on the forearm of the right hand (Fig. 1a) on the 30 healthy, Caucasian skin type, male volunteers within the 19-21 age range. Here the volumetric flow is largely related to the metabolism of the tissue, rather than to the thermoregulatory control (for example, the skin of the palmar surface of the fingers). The instruments for the registration of perfusion (by LDF method) and fluorescence spectra (by FS method) during the occlusion test, used "LAKK" series equipment production by SPE "LAZMA" Ltd (Russia)[9, 10]. The laser Doppler flowmetry device "LAKK-02" (wavelength sensing – 1064 nm) was used for perfusion measurement ($I_m$ – index of microcirculation) and subsequently to estimate the nutritional blood flow level ($I_{mn}$). A fluorescence spectrum was registered using the fluorescence channel of "LAKK-M" system, with two wavelengths excitation of endogenous NADH and FAD fluorescence at 365 nm and 450 nm respectively. For simultaneous recording of optical fibre probes, parameters were recorded at one study site by combining fibres of the "LAKK-02" and the "LAKK-M" devices.

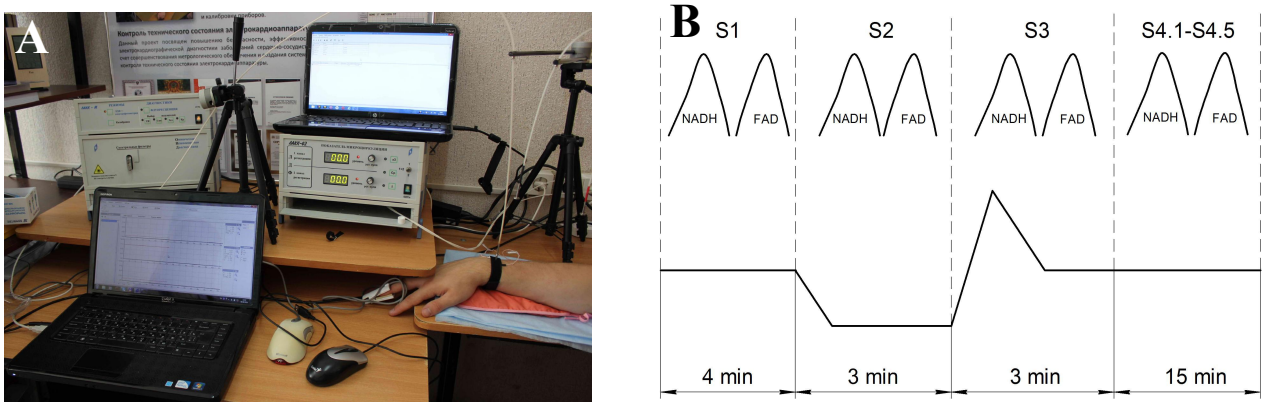


Figure 1. Experimental setup (a) including "LAKK-M" and "LAKK-02" systems and a timing chart of the experiment (b), where S1-S4 are respective stages.

The study consisted of 4 stages (Fig. 1b): state of rest (4 min of perfusion record), artificial ischemia (occlusion during 3 min), reactive hyperemia (3 min) and recovery (relaxation during 15 min). An occlusion test (OT) was performed according to standard procedure with 220 mm Hg pressure on the upper arm (typical example for the LDF-gram during OT shown on the Fig. 2). During the first three stages, one-time measurements of NADH (peak at 460-470 nm) and FAD (peak at 510 nm) fluorescence were taken at two excitation wavelengths (Fig. 3). Paired measurements were taken every 3 minutes during the fourth, relaxation stage. Total time of one experiment was 25 min.

RR was calculated as the ratio of the normalized amplitudes of NADH to FAD according to the 4 approaches:

$$RR_1 = \frac{I_{em\_NADH}}{I_{em\_FAD}}; \tag{1}$$

$$RR_2 = \frac{k_{NADH}}{k_{FAD}}, k = 1 + \frac{I_{em} - I_{ex}}{I_{em} + I_{ex}}; \tag{2}$$

$$RR_3 = \frac{I_{em\_NADH}}{I_{em\_FAD}} \cdot \frac{I_{ex\_FAD}}{I_{ex\_NADH}}; \tag{3}$$

$$RR_4 = \frac{I_{em\_FAD}}{I_{em\_NADH} + I_{em\_FAD}}, \tag{4}$$

where $I_{em}$ is the intensity of fluorescence peak, and the $I_{ex}$ is the intensity of laser peak. The tissue fluorescence contrast range coefficient $k$ is used for fluorescence estimation.

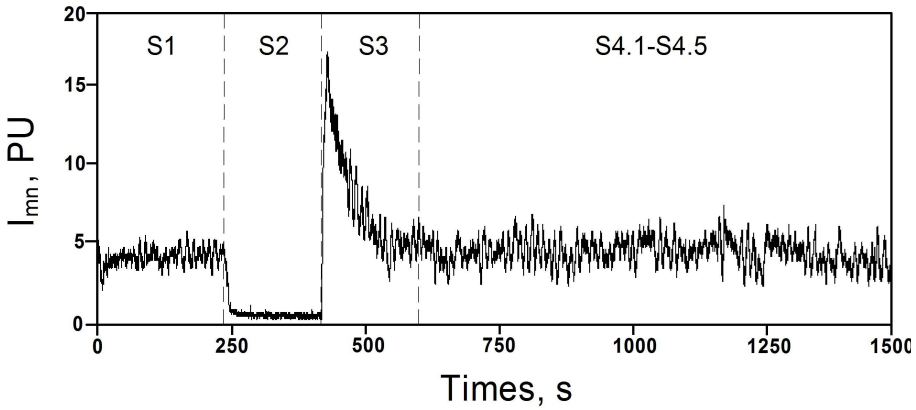


Figure 2. Typical example for the LDF-gram during experimental study, where S1-S4 – respective stages.

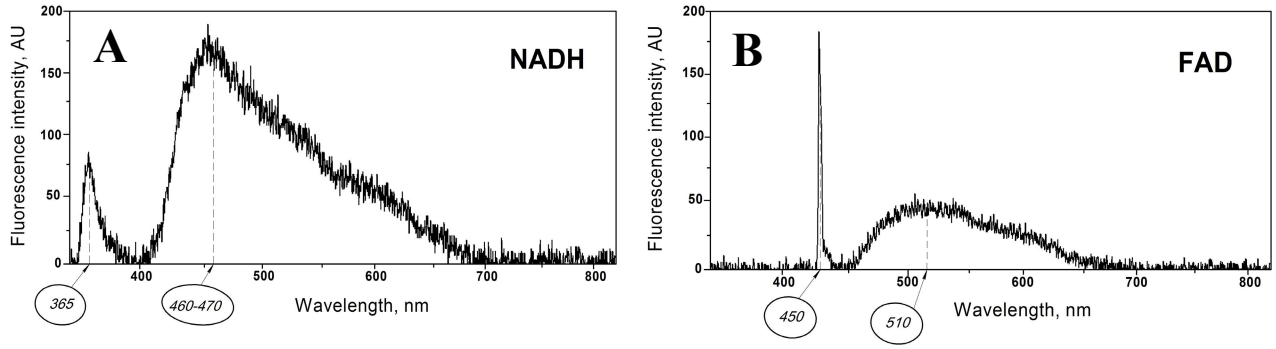


Figure 3. The typical example of fluorescence spectra of endogenous fluorophores – NADH (a) and FAD (b) – on the right forearm of Caucasian skin type.

In order to assess the perfusion oscillatory component for the nutritive blood flow calculation (according to the methodology[11]), spectral wavelet analysis of oscillations was used (software LDF 3.0.2.384, "LAZMA", Russia). This program uses a continuous wavelet transform, with the Morle complex valued wavelet being used as the analyzing wavelet[12]. The study was performed at an ambient temperature of 21-22°C in a sitting position after a 30 min rest.

## 3. EXPERIMENTAL RESULTS AND DISCUSSION

Formulas (1-4) were used for the calculation of the redox ratio. Formula (3) was selected for further analysis of the data. In our opinion, only it can qualitatively analyze the connection between redox and blood flow in the stages of occlusion and hyperemia, as it best agrees with the theory and exhibits less scatter in the data.

The results obtained from calculating the redox ratio were further divided into three groups on the basis of their reaction to the occlusion test. These groups were the: reduction of redox ratio (Fig. 4), increase of redox ratio (Fig. 5) and no change in redox ratio (Fig. 6).

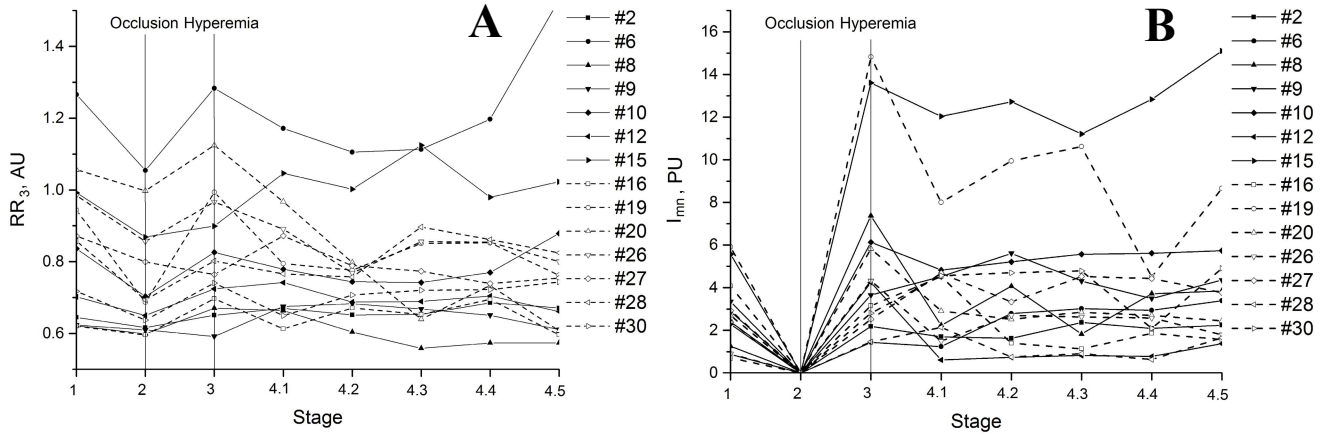


Figure 4. Relationship between redox ratio (a) and nutritive blood flow (b) registered on the forearm of 14 volunteers with a typical response of reduction of the redox ratio as a result of the occlusive test.

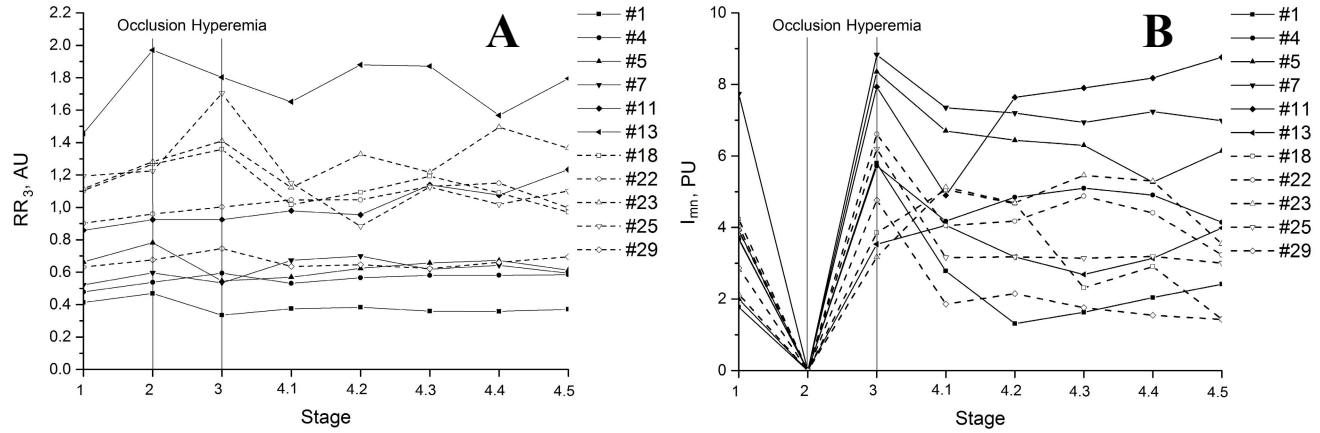


Figure 5. Relationship between redox ratio (a) and nutritive blood flow (b) registered on the forearm of 11 volunteers with a typical response showing an increase of the redox ratio as a result of the occlusive test.

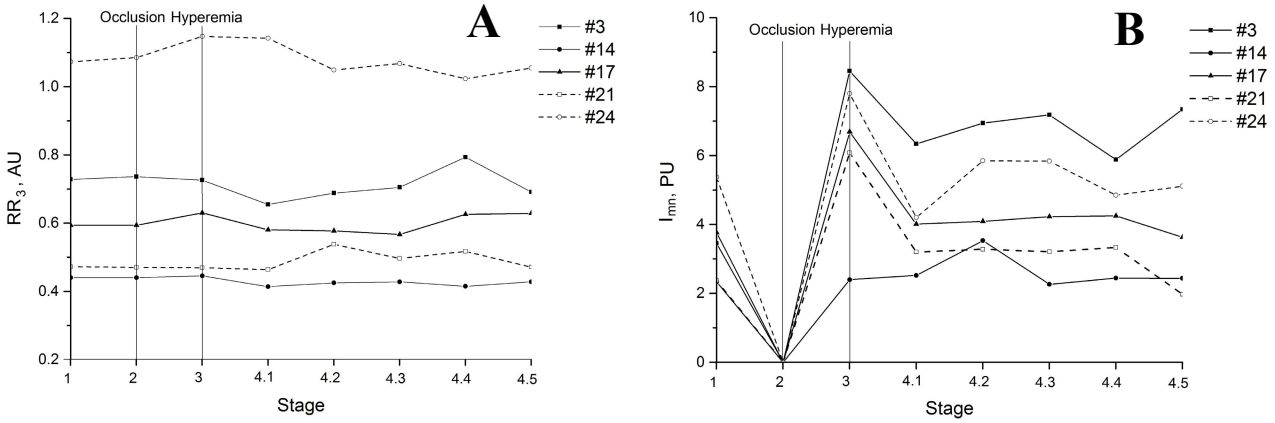


Figure 6. Relationship between redox ratio (a) and nutritive blood flow (b) registered on the forearm of 5 volunteers with a typical unchanging response of the redox ratio as a result of the occlusive test.

Figure 7 shows the redox ratios dependence on the nutritive blood flow based on the averaged data of the previously described 3 groups of volunteers: 1 – increased RR during occlusion group, 2 – decreased RR during occlusion group and 3 – no change in RR during occlusion group.

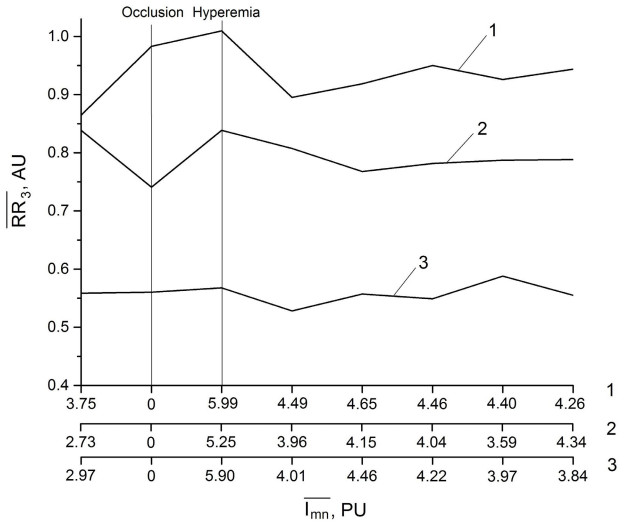


Figure 7. The redox ratios dependence on the nutritive blood flow based on the averaged data of the three volunteer groups.

As is known, metabolic activity is closely tied to the nutritive blood flow in most organs and tissues in accordance with the physiological law of "delivery-consumption" of oxygen. For example, rising tissue metabolism as seen during muscle contraction or as a result of neuronal activation, leads to an increase in blood flow.

It is also know that under hypoxic conditions, a build-up of NADH acts as one of the early signs of anaerobic glycolysis activation. Thus, during ischemia, a qualitative change of the redox ratio should theoretically be observed. This observed change is presented in figures 4-7.

To evaluate the adaptive capabilities of the volunteers we proposed a criterion linking the dynamics of redox ratio change during the occlusion and relaxation stages. The changes in redox ration during each stage was considered as the difference $RR_3$, taken from readings prior to and during occlusion and during occlusion and during the relaxation stage (formulas 5-6):

$$\Delta RR_{before} = \left| RR_{S1} - RR_{S2} \right|, \tag{5}$$

$$\Delta RR_{after} = \left| RR_{S2} - RR_{S4.1} \right|. \tag{6}$$

The difference between the two redox ratio changes serves as a direct criterion:

$$AM = \left| \Delta RR_{before} - \Delta RR_{after} \right|. \tag{7}$$

We propose that judgment of and organism's adaptation potential to be done based on the value of the final parameter. Thus, the lower this indicator, the higher the adaptation potential.

For example, volunteer 6 of the first group (Fig. 4), exhibits large oscillations in metabolic activity (RR). This process is characterised by a large index of the proposed criterion, namely AM = 0.1. However, volunteer 2 of the same group, does not exhibit similar oscillations, which corresponds to a very low value of the proposed criterion AM = 0.003.

It is worth noting that in the recovery phase we observed two types – rapid recovery of the normal metabolism level (RR) and a slow recovery or maintained high level of metabolism compared with baseline values. The second option shows a decline in adaptive capabilities of MTS.

It is also worth noting that for many investigated volunteers, a high dynamic of RR recovery was observed in the first stage of relaxation. This could correspond to the young age of the volunteers and their healthy status.

## 4. CONCLUSION

With the help of non-invasive LDF and FS methods, we have showed the relationship between the nutritive blood flow and the redox ratio in human skin in terms of physiological rest. The speed of change of metabolism in the phase of occlusion and reperfusion as well as the phase of recovery duration may be the criteria for adaptive capabilities of MTS, which has practical significance for physiology and medicine.

Preliminary results in the study of a complex approach to diagnosis of the state of biological tissue were obtained. A positive relationship between the nutritive blood flow in the microcirculatory channel and RR of skin tissue is observed. The positive results of these experiments suggest the need to continue further studies, as this will result in the improvement of the methodological and the instrumental base for use of fluorescence spectroscopy technology in medicine.

## ACKNOWLEDGMENTS

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