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# Direct optical generation of singlet oxygen in the regulation of vascular tone

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## ABSTRACT

An approach to visualization of the vascular bed with the possibility of assessing changes in blood filling and identifying diagnostically significant periodic changes in the signal by analyzing speckle images is proposed. The effect of singlet oxygen by direct excitation of an oxygen molecule by 1267 nm laser radiation on changes in the vascular bed parameters was studied using this approach.

**Keywords:** singlet oxygen, direct optical generation, 1267 nm, vascular bed, PPG, high-resolution speckle imaging.

## 1. INTRODUCTION

Oxygen and its partially reduced chemical products known as reactive oxygen species (ROS) have important regulatory and signalling functions. ROS, along with reactive nitrogen species, play a special role in the regulation of vascular tone<sup>1</sup>. The singlet form of oxygen is one of the ROS. It is known, the vascular bed response to the action of singlet oxygen is characterized by multidirectional reactions and can manifest itself in the form of vasodilation and vasoconstriction of vessels<sup>2,3</sup>.

The important role of singlet oxygen in the shutdown of the vascular bed feeding tumor tissues during photodynamic therapy of oncological and non-oncological tumor diseases with the use of photosensitizers (PS) has been revealed<sup>4</sup>. However, the high toxicity of PS (complications and possible side effects, effects on healthy tissues, the excretion time duration from the body) does not allow us to conclude about the exceptional effect of singlet oxygen on the vascular bed<sup>5</sup>.

To date, in addition to the classical mechanism of singlet oxygen generation using PS, direct optical generation of singlet oxygen has become possible<sup>6</sup>. The possibility of oxygen molecule excitation by certain wavelength light and regulation of singlet oxygen production by changing the light intensity and exposure time is of undoubted interest for fundamental and practical medicine. In particular, the influence of singlet oxygen not mediated by the influence of PS on the state of the vasculature remains unresolved at the moment and requires study.

In addition, it seems promising to develop approach to visualization of the vascular bed with the ability to assess changes in blood supply and identify periodic changes in the signal, related to breathing and heartbeat.

## 2. MATERIAL AND METHODS

The proposed approach to visualization of the vascular bed consists in simultaneous registration of backscattered intensity images of the skin area illuminated by non-coherent (525 nm) and coherent (660 nm) light sources using a large aperture optical system and color CMOS camera (Fig.1a). The use of combined illumination makes it possible to obtain focused images of the skin in the green channel and use them to superimpose speckle interference patterns obtained in the red channel. In addition, light sources with different penetration depths into biological tissues make it possible to visualize superficial and deeply located blood vessels<sup>7</sup>. During the studies the side illumination is installed so as to achieve maximum uniformity of illumination of the study area.

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Processing of the resulting images includes splitting data into RGB channels, compensation for uneven the camera sensor sensitivity, images contrast enhancement, compensation for uneven illumination field, full-frame and local superimposition of capillary images, calculation of photoplethysmography (PPG) signals and restoration of vessels maps (525 nm) and vessels perfusion maps (660 nm) by Fourier spectrum modulation calculation.

Additionally, the analysis of periodic changes in signals is carried out. This analysis is important from the perspective of a possible study of changes in local amplitudes of blood pulsations (assessment of blood filling) before, during and after the generation of singlet oxygen. In addition, the analysis of changes in the signal modulated by breathing and heartbeat will allow us to assess the contribution of systemic changes to the recorded signals.

Direct optical generation of singlet oxygen is carried out at a wavelength of 1267 nm (Fig. 1a) using the developed device. The choice of laser radiation parameters (power and exposure time) is made taking into account the optical properties of biological tissue (skin) and its hemodynamic characteristics. The results of modelling and thermal imaging measurements<sup>8</sup> show that 1267 nm laser radiation with a power of 50 mW causes minimal heating of the studied area (Fig. 1b).

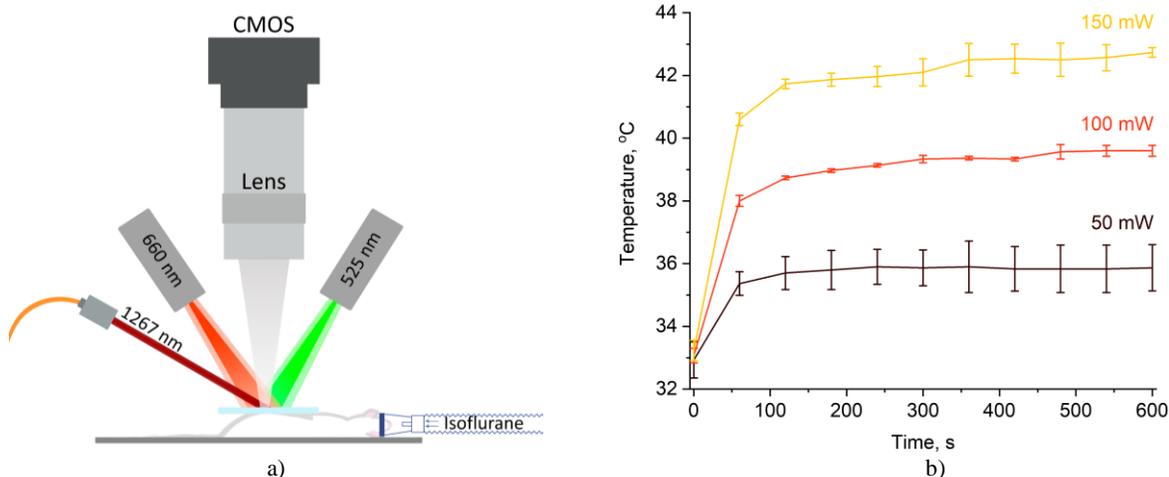


Figure 1. (a) Scheme of the experimental setup; (b) the dependence of the study area heating on the laser radiation power and exposure time.

Using this approach, the effect of direct optical generation of singlet oxygen on changes in the parameters of the vascular bed was studied. The studies were carried out on Wistar rats aged over 1 month weighing 200-300 g with previously removed hair in the gluteal and femoral regions. The animal work was approved by local Ethical Committee. During the experiment, the rats were under inhalation anesthesia. Isoflurane was used as an anesthetic, which was delivered using the R540 Mice&Rat Animal Anesthesia Machine system. This anesthetic provides a rapid onset of general anesthesia, a quick exit and causes the least depression of the cardiovascular system. The dose was selected taking into account the age and weight of the animal.

To reduce the effect of skin displacement and the probability of image defocusing and reduce local displacement in the horizontal plane, the images were registered through a glass plate slightly touching the skin<sup>9</sup>. The transparency of the skin was increased by applying immersion oil between the skin and the glass.

Taking into account the small diameter of the vessels and the high velocity characteristics of the blood flow of rats, measurements were carried out with a frame rate of 250 frames per second and a frame size of 600×600 pixels. The total magnification multiplicity of system the was 3.5, the size of the field of view was ~1×1 mm. The recording of the experiment was carried out continuously and included three stages: before exposure for 1 min, during exposure with considering the dose, 10 min after exposure.

### 3. EXPERIMENTEL RESULTS AND DISCUSSION

The results of changes in the PPG signal during the study under the exposure of 1267 nm radiation with a dose of 50 J/cm<sup>2</sup> are shown in Fig. 2.

Fig. 3 presents vessels maps at 525 nm (a – without and b – with Fourier spectrum modulation calculation) and vessels perfusion maps at 660 nm (c) before (upper row), during (middle row) and 10 min after (lower row) exposure to a dose of 50 J/cm<sup>2</sup>.

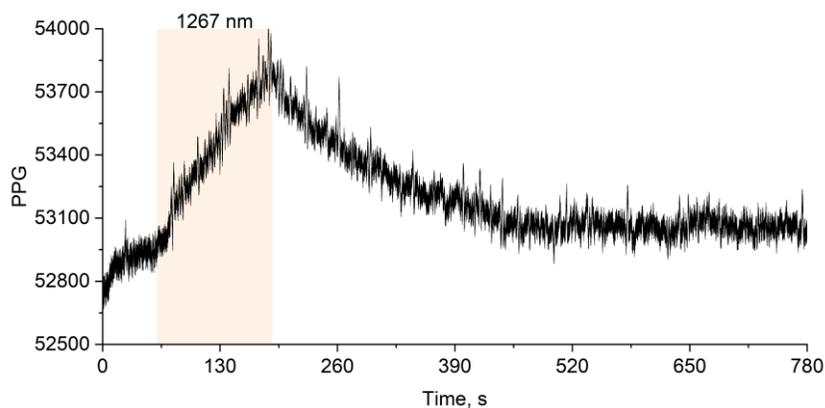


Figure 2. The PPG signal during the study under the exposure of 1267 nm radiation with a dose of 50 J/cm<sup>2</sup>.

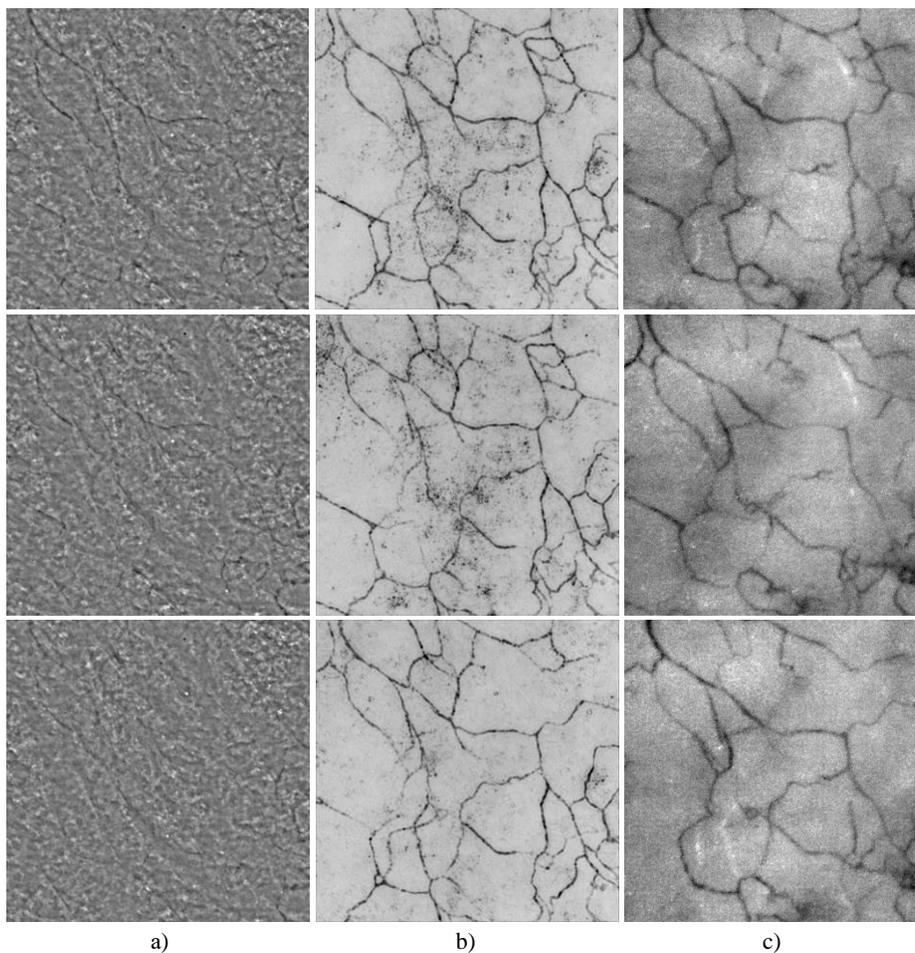


Figure 3. Vessels maps at 525 nm (a, b) and vessels perfusion maps at 660 nm (c) before (upper row), during (middle row) and 10 min after (lower row) exposure to a dose of 50 J/cm<sup>2</sup>.

As can be seen from the presented data, direct optical generation of singlet oxygen during exposure at a wavelength of 1267 nm to a dose of 50 J/cm<sup>2</sup> leads to a change in the capillary bed, namely, to a decrease in blood filling (see PPG results) and vasoconstriction and shutdown of blood vessels (this is clearly visible on the processed speckle images, Fig. 3c). Reduced blood flow may be associated with noradrenaline-mediated vasoconstriction due to Ca<sup>2+</sup>-independent noradrenaline release from the prejunctional site of adrenergic neurotransmission, caused by singlet oxygen<sup>10</sup>.

It is important to note, synthesized vessels perfusion maps using 660 nm channel (Fig. 3c) were built for 6000 frames with localization of 24 s. When studying fast processes, localization in a shorter period of time may be required, while the image quality will be reduced. Fig. 4 shows the vessels perfusion maps calculated with localization of 4 s (1000 frames) (a), 2 s (500 frames) (b) and 1 s (250 frames) (c).

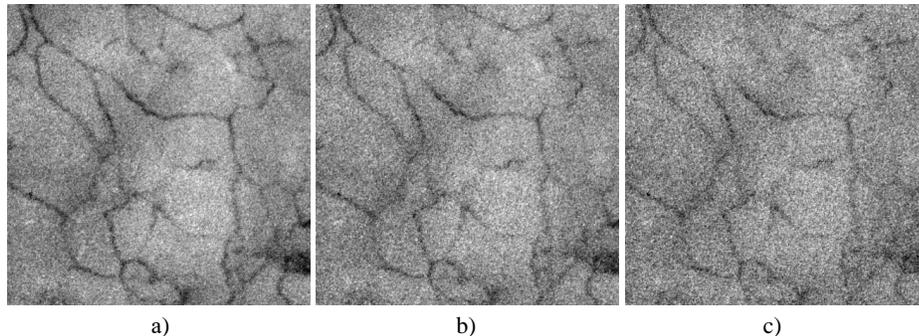


Figure 4. The vessels perfusion maps calculated with localization of 4 s (1000 frames) (a), 2 s (500 frames) (b) and 1 s (250 frames) (c).

As can be seen, the proposed approach allows to identify and analyze processes with a frequency of change up to 1 Hz without significant deterioration in the quality of vessels perfusion maps.

#### 4. CONCLUSION

The proposed approach and the implemented setup allow for continuous, long-term, high-speed recording of backscattered intensity images. Image analysis allows to reconstruct a PPG signal and, for any time point of interest, restore vascular maps and vascular perfusion maps from an existing speckle images set, assessing their activity, including the study of rapidly changing processes. The developed method made it possible to study the effect of singlet oxygen generated by laser radiation on the vascular tone.

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