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Experimental study of the influence of blood flow on the fluorescence signal of biological tissue

V. Dremin[1], A. Zherebtsova[1], I. Novikova[1], E. Zharkikh[1], E. Zherebtsov[1], A. Dunaev[1], K. Litvinova[2], I. Rafailov[2], A. Krupatkin[3], V. Sidorov[4]

[1]Biomedical Photonics Instrumentation Group, Scientific-Educational Centre of "Biomedical Engineering", State University – Education-Science-Production Complex, Oryol, Russia
[2]Optoelectronics and Biomedical Photonics Group, Aston Institute of Photonic Technologies, Aston University, Aston Triangle, Birmingham, UK
[3]Priorov Central Research Institute of Traumatology and Orthopaedics, Moscow, Russia
[4]SPE "LAZMA" Ltd, Moscow, Russia

Introduction

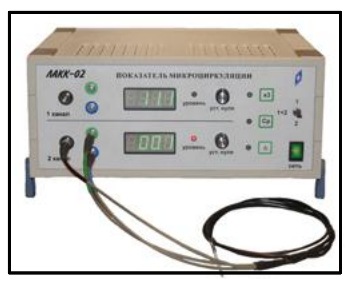
In recent years, fluorescence spectroscopy (FS) has been successfully used in studies of biological objects at the micro level. However, in general, one of the main problems with this method so far, is the correct interpretation of the data. It is known that changes in blood flow can affect the fluorescence spectra: it may result from signal attenuation due to the absorption capacity of the blood and the change in the concentration of fluorescent coenzyme occurring during concomitant changes in metabolism.

The aim of research

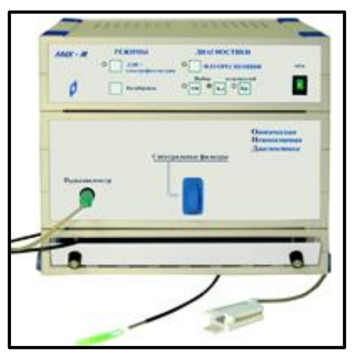
To experimentally study the effects of blood flow on the fluorescence signals in biological tissues and theoretically describe this effect.

Experimental method and equipment

Laser Doppler flowmetry "LAKK-02"



Fluorescence spectroscopy "LAKK-M"



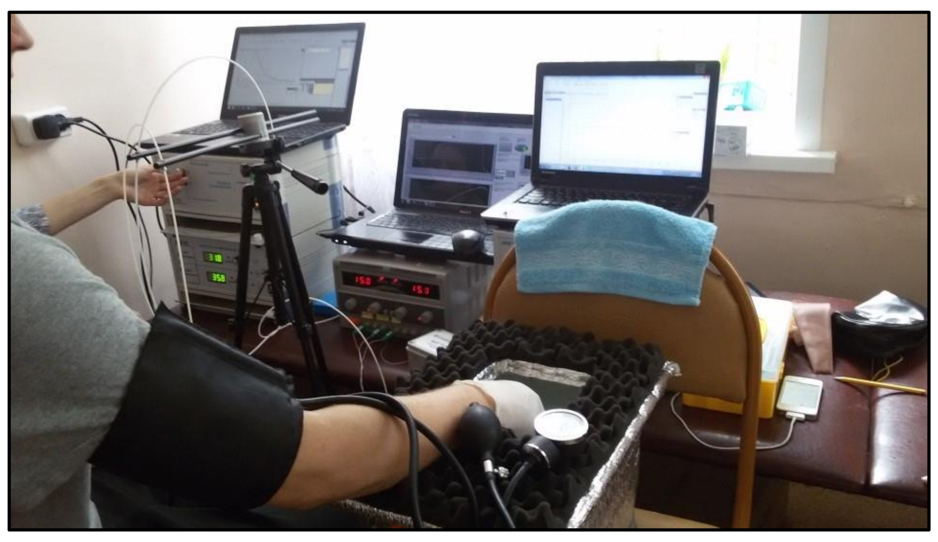
Thermometry 2-channel temperature sensors:

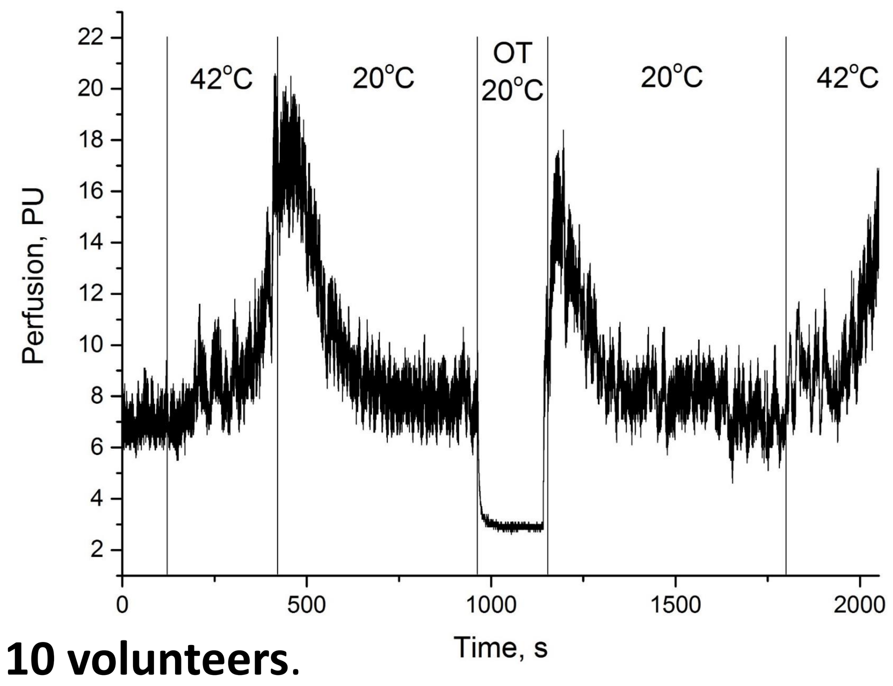
Special tooling designed and 3D printed

To evaluate the intensity of blood flow, perfusion was recorded by laser Doppler flowmetry (LDF) with a "LAKK-02" laser analyzer. The fluorescence spectrum of biological tissues was detected by means of the "LAKK-M" complex FS channel (SPE "LAZMA" Ltd, Russia) for 2 excitation wavelengths – 365 and 450 nm, respectively.

Special tooling designed and 3D printed, allows the placement of the measuring LDF and FS channel fibre along the arm, as well as the ability to secure additional 2-channel temperature sensors.

The Concept

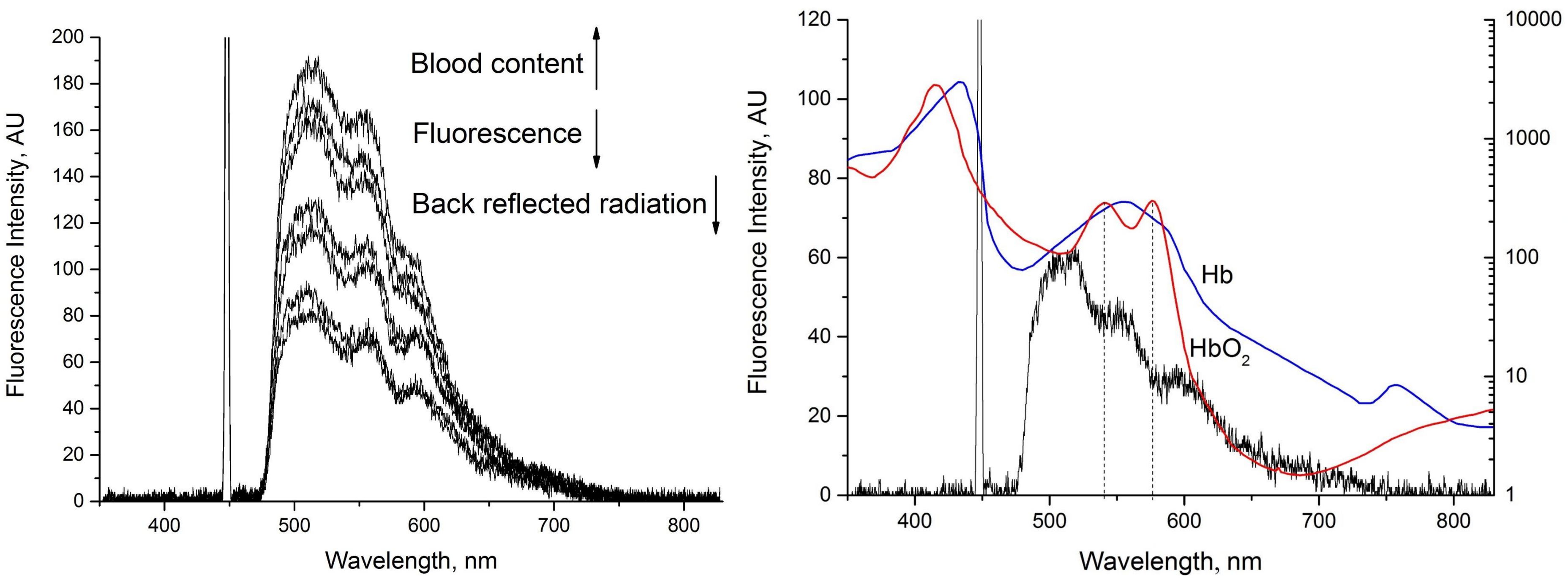




Experimental study

A total of **37 experiments** were performed on **10 volunteers**.

Experimental studies were conducted on the palmar surface of the skin of the middle and ring fingers of the right hand of healthy volunteers. The idea of the research was the implementation of provocative actions on blood flow through changes in extremity temperature and application of the brachial arterial occlusion cuff with pressure.



Typical examples of blood interference in fluorescence spectrum

Mathematical model

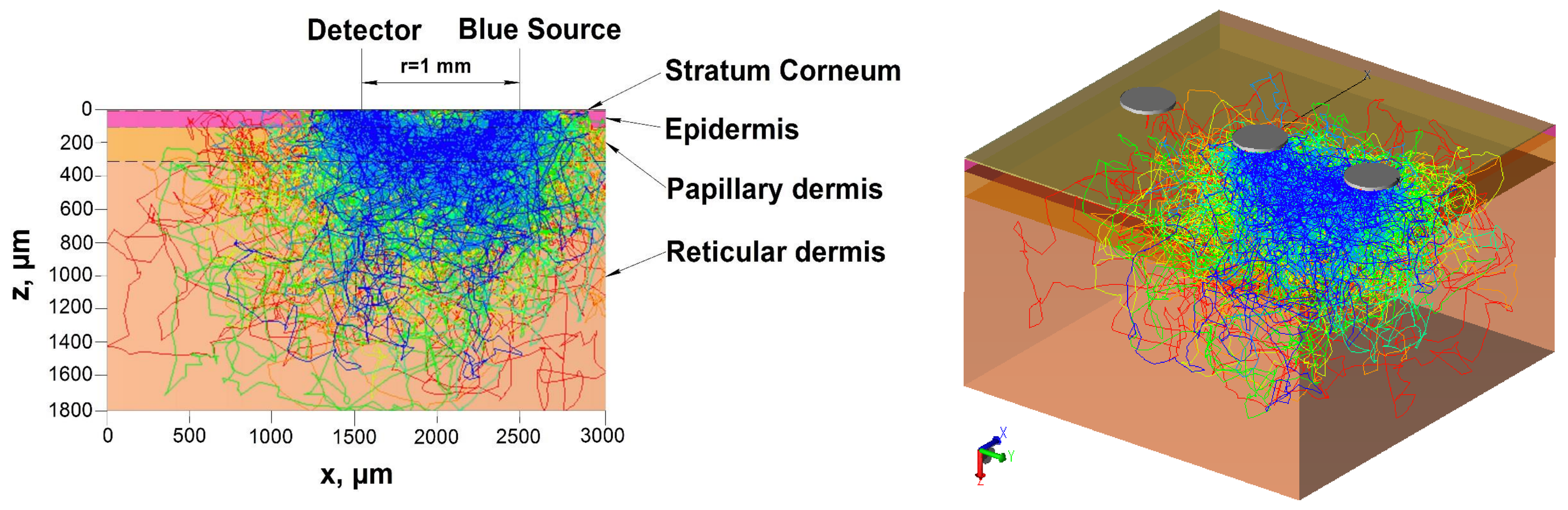
Necessary to develop mathematical models and methods of fluorescence spectra correction. Creating analytical models is very difficult due to the complexity of solving the problem, even for simple cases. Numerical methods, including the probabilistic Monte Carlo, are often used to solve the problems of light propagation in biological tissue. In this work we propose one possible implementation of this method, as well as a simplified correction model based on Beer's law.

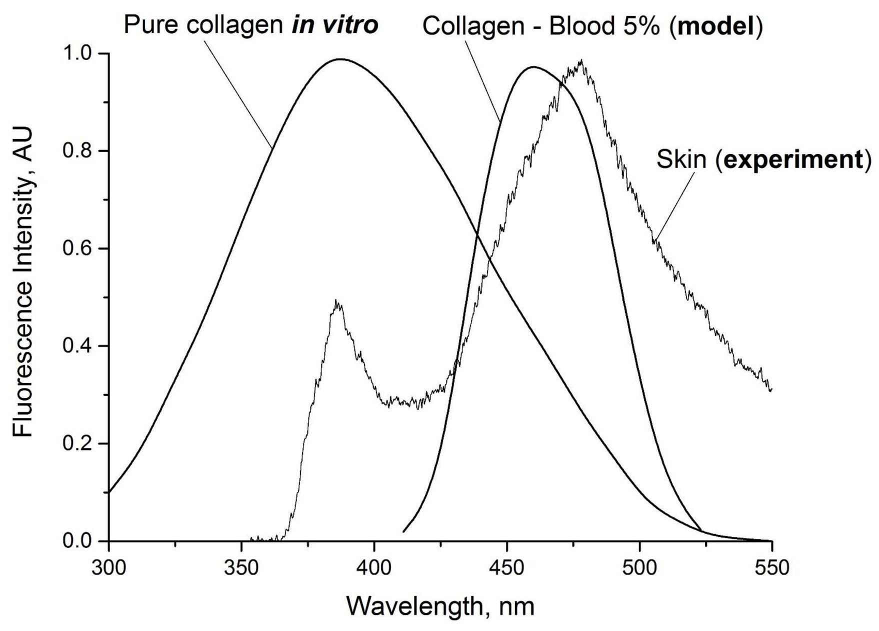
Results and Discussion

Experimental study

All volunteers displayed a high **inverse correlation (~0.4-0.8)** between registered back reflected radiation and the intensity of fluorescence from biological tissue perfusion and temperature for both wavelengths. Thus, we can assume that factors affecting the absorption of the blood change the fluorescence spectra of biological tissue.

Mathematical model (Numerical Monte Carlo modeling)





An example of modelling the impact of blood on the fluorescence spectrum

Based on the developed optical model and further computer simulations, the general distribution of light within tissue can be predicted. This can be invaluable in a variety of non-invasive optical methods of investigation, ultimately leading to potential new avenues of modelling and diagnostics.

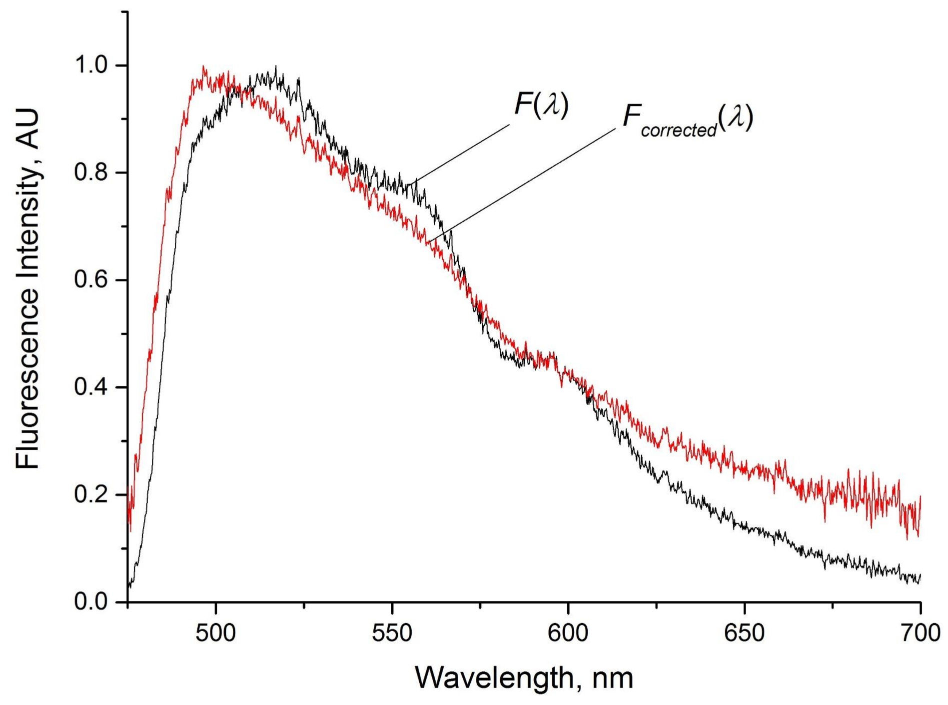
Correction of fluorescence spectra using data from diffuse reflectance spectroscopy (DRS)

Using the spectral data provided by the DRS, tissue convolution effect can be extracted and used to correct the fluorescence spectra. In this work, we use an approximation of Beer's law to compute the light absorption coefficient of blood.

Supposing, the majority of photons undergo a very small number of scattering events before being collected by the probe. This fact satisfies the applicability of Beer's law in turbid media.

Simplified correction model – Beer's law

$$F_{corrected}(\lambda) = \frac{F(\lambda)}{\exp[\frac{l_F}{l_R} \cdot \ln(R(\lambda))]}$$



Conclusion

The results obtained must be considered in fluorescence spectroscopy research to determine the concentrations of various fluorophores in biological tissue and the development of new diagnostic criteria and normalizing factors. Also, the results of these experiments indicate a need for further studies, since this generally leads to improved methodology and instrumentation technology for use in medical fluorescence spectroscopy.

Acknowledgements

This work were supported by basic part of the state task of the Ministry of Education and Science of the Russian Federation (GZ №310), by the grant of State University ESPC (VK-3-2013) and by the Russian Foundation on Innovations U.M.N.I.K.