Fluorescence imaging with endogenous contrast is widely used for in vivo biomedical diagnostics due to its non-invasiveness, molecular specificity and sensitivity. Extensive research in this area resulted in the emergence of the classical paradigm for the interpretation of the autofluorescence signal from biotissues, including the origin of chromophores and fluorophores, the formation of their photophysical properties and interrelation between optical properties and biochemical processes in the human organism. At the same time, recent results demonstrate a number of effects, which can not be explained in the scope of the classical framework, thus stimulating discussion of challenges and perspectives of fluorescence molecular imaging. The lectures will highlight the latest advances in this area due to technological innovations and underline the major challenges for future investigations.

The short course will be sub-divided into two parts:

1. Autofluorescence as a versatile tool for biomedical diagnostics

The first part of the course will introduce the research area of autofluorescence. The questions of the origin of chromophores and fluorophores in the human organism, the basics of their photophysics, i.e., how the fluorescence parameters depend on the molecule’s structure and its microenvironment, will be discussed. A classical list of fluorophores will be presented with an emphasis on the interrelation
of each fluorophore and certain biomedical diagnostics task. A brief review of fluorescence imaging methods will be provided, including confocal and multiphoton microscopy, fluorescence diffuse optical tomography and fluorescence lifetime imaging. The power of autofluorescence in biomedical diagnostics will be illustrated by several recent examples with a focus on how the source of fluorescence can be identified and which biochemical information can be extracted from the endogenous fluorophores.

2. Terra incognita in autofluorescence

In the second part of the course, it will be shown that the classical interpretation of autofluorescence formation lacks understanding of several important phenomena. Firstly, the origin of ubiquitous fluorescence emission from tissues in the red and NIR spectral ranges is unknown. This effect is extremely significant for biophotonics due to its relevance to background formation in Raman spectra and predictive capabilities for detecting tumor margins, and current views on this subject will be discussed. Secondly, a lively discussion occurs in the literature about the emergence of visible fluorescence in peptides and proteins following their aggregation, as well as during self-assembly of metabolites in vitro and in vivo. The origin of this effect is supposed to originate from charge transfer or proton transfer processes, excitonic interactions or formation of oxidation products. A critical review of these hypotheses will be presented in the lecture. These and some other recent findings will be used to demonstrate that the research area of endogenous fluorescence is continuously evolving, and despite decades of research, there is still plenty of space for developing novel diagnostic methods based on endogenous fluorescence contrast.

Learning objectives

This course will provide the participants with:

→ basic understanding of autofluorescence signal formation in biotissues based on the classical list of endogenous chromophores and fluorophores in the human organism;

→ basic knowledge of which information can be extracted from the autofluorescence signal and how it can be used for diagnostics; understanding of the principal, advantages and limitations of this method;

→ getting familiar with current challenges and advances in understanding the origin of endogenous fluorophores, including emission in the red and NIR spectral regions.

Intended audience

Undergraduate, Master and PhD students, postdoctoral trainees, and research scientists in the areas of physics, engineering and biomedicine.
Course level
Intermediate

Short Course duration
Two hours

Instructor

Dr. Evgeny A. Shirshin’s research focuses on development of novel methods for intravital optical imaging for biomedical diagnostics and fundamental study of photophysical processes responsible for optical properties of molecules in the human organism. Dr. Shirshin is a Senior Researcher at the Faculty of Physics of M.V. Lomonosov Moscow State University and Leading Researcher in the Moscow State University Clinics. He also leads the research in biophotonics in the Institute of Spectroscopy of the Russian Academy of Sciences. He received Master’s Degree in Physics in 2008 and a PhD degree in Laser Physics in 2011 from the Moscow State University, Russia. Dr. Shirshin is an author of over 50 peer-reviewed publications, and his research activities are funded by grants from the Russian and international funding agencies.