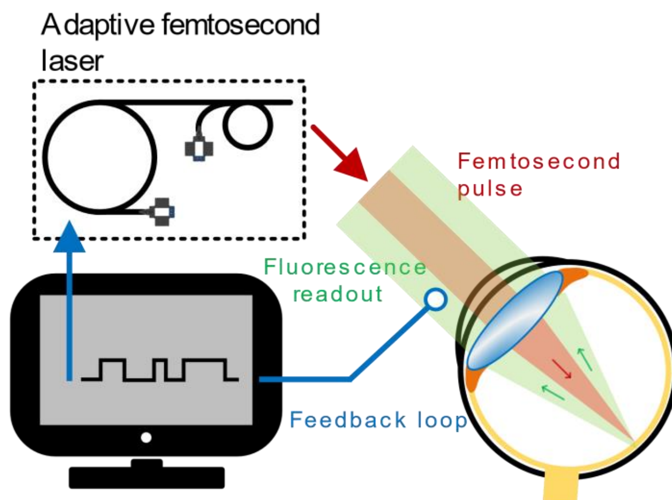
The interaction of light with matter is the foundation of many phenomena - it allows us to study the fundamental properties of unknown materials, investigate the properties of light, and enable many practical applications. One such example is the formation of contrast in optical imaging. The way light is scattered, refracted, absorbed, or transmitted allows visualization of various structures and is the basis of optical microscopy, optical coherence tomography, or fluorescence microscopy. These methods allow studying the structure or composition of various objects - biological tissues, low-dimensional materials, semiconductor structures, or pharmaceuticals. Due to the possibility of optical sectioning, some of these methods allow to look deep into the investigated structures, importantly, in a non-invasive and non-destructive way.

Interaction efficiency is particularly critical when using light in biomedical imaging. It is essential for delicate tissues such as the eye, skin, or brain. In this case, the interaction of light with matter is necessary to obtain information, but it can also act in a way that modifies or destroys the examined environment. The mechanism can be, e.g., phototoxicity or thermal interactions. Therefore, the efficient use of each photon that is sent to the tested object is extremely crucial. It is essential that the efficiency of this interaction is as high as possible so that a clear tissue response can be obtained with as little excitation power as possible. It is also very important for the light to go exactly where we want and for the interaction with the tested object to be as well localized as possible, i.e., occurring in the place we study and with the substances, we are currently interested in. An additional difficulty is the great variety of objects under investigation, both in terms of geometry and molecular composition. For example, during ophthalmic examinations, there may be differences in eye size, refractive power, or the presence of diseases (e.g., glaucoma) between individual patients, which drastically affects how light will propagate through the object under study.

The proposed project aims to solve a problem that occurs in ophthalmic imaging, specifically in two-photon fluorescence imaging of the human fundus. It is a new method that allows imaging the distribution and concentration of fluorophores located in the retina and retinal pigment epithelium, which carries crucial diagnostic information about vision diseases and can be used during therapy monitoring. Two-photon excitation of fluorescence is a novel approach that carries many advantages, such as the ability to excite fluorophores that cannot be excited otherwise (absorbing in the UV range), greater patient comfort, and a wider spectral range of detection. On the other hand, it requires the use of ultrashort laser pulses in the near-infrared, which imposes very restrictive limits on the power of the laser beam used. The problem lies in the low efficiency of fluorescence excitation in such conditions, the difficulty in matching the excitation wavelength to the fluorophores, and the high biological variability of the objects under study.

We wish to answer how far it is possible to control the properties of light to intensify two-photon fluorescence excitation in a precisely localized and individualized manner, closely tailored to the object under study. Biological systems are characterized by a wide variety of fluorophores (varying in absorption spectrum), scattering, and dispersion, which results in pulse stretching, peak power reduction, and consequent decrease of contrast. Optimization of fluorescence intensity is possible by appropriately matching the spectrum of the excitation pulse to the fluorophore under study (as well as transmission through preceding tissues) and selecting the temporal properties of the pulse such that the shortest possible pulse reaches the tissue under study. The approach we propose is to modulate the spectral and temporal parameters of the excitation pulses, appropriately controlled by an algorithm that optimizes the signal sought. To this end, we plan to develop a novel programmable source of ultrashort pulses of light (on the order of femtoseconds, i.e., 10^{-15} seconds) that could adaptively tune its parameters to maximize the signal of induced fluorescence. With future applications in mind, we plan to develop the laser source using fiber optics and nonlinear optics. We propose to generate pulses using a femtosecond fiber oscillator and then extend it to the required spectral range (650 - 950 nm) by generating supercontinuum radiation in nonlinear optical fibers. Then, a spectral phase modulating system coupled with an optimization algorithm will then be developed using an electronically controlled spatial light modulator.



This research is motivated by applications in ophthalmology. However, its context is much broader and may be relevant wherever the efficiency of fluorescence excitation is crucial, such as in neuroscience or dermatology. The proposed project involves creating a tool and acquiring new knowledge in the adaptive shaping of ultrashort laser pulses. It will allow improving signal quality and contrast in two-photon fluorescence imaging, translating into richer information and potentially opening new diagnostic possibilities.