Characterization of sensitivity of cones and rods to two-photon stimulation

Two-photon vision is a recently discovered mechanism that expands the range of electromagnetic radiation that can be perceived by the sense of sight. It is based on two-photon absorption, whereby visual pigment molecules simultaneously absorb two photons of twice smaller energy instead of the one photon of energy doubled. Therefore, laser beams perceived due to two-photon vision have a color close to half their wavelength. Two-photon absorption is a rare process compared to single-photon absorption. Observing it at the macroscale requires pulsed lasers that emit momentarily large photon fluxes, needed for the process to occur frequently enough. It was surprising to discover that it occurs under conditions safe for the eye. However, this fact is related to the exceptional sensitivity of our vision, which can detect even single photons under the right conditions.

Previous studies of two-photon vision have focused on ophthalmic applications, particularly for microperimetry, a visual field test used, i.a., in diagnosing glaucoma. The two-photon process allows more precise stimulation of the retina and more accurate determination of the so-called visual threshold, i.e. the lowest brightness of the stimulus that could be perceived. However, two-photon visual perception may also find applications in other fields, such as augmented reality displays. Therefore, further characterization of the phenomenon is needed to develop standards for measuring the brightness of two-photon sources and enable future engineers to design devices that take advantage of this effect.

Humans have several types of photoreceptors: three types of cones for sharp daily color vision and sensitive rods to detect objects under low-light conditions. Each photoreceptor type contains visual pigments with a slightly different structure and absorption spectrum. The photoreceptors' morphology is, to some extent, different as well. This project aims to answer the question: do these differences affect the probability of two-photon absorption and thus modify the effectiveness of two-photon stimulation of individual photoreceptor types? What is the exact shape of the spectral sensitivity of the different photoreceptor types to this process? How do the parameters describing two-photon visual perception differ from one-photon perception? Are the critical flicker frequencies different, which would affect the refresh rate of the displays? What is the two-photon contrast sensitivity?

Our recent results suggest that some differences do indeed exist. The color of a two-photon stimulus is not exactly the same as its half-wavelength one-photon counterpart. The difference in the absolute maximum sensitivity of cones and rods for two-photon vision is an order of magnitude smaller than for normal vision. The pupillary light reflex is also significantly smaller for a two-photon stimulus. The retinal sensitivity maps for two-photon vision are flatter than the maps obtained for one-photon vision under the same conditions. All of these results suggest that two-photon stimulation of cones and rods differs in efficiency, and cones appear to be more prone to this process.

Two-photon stimulation of photoreceptors will be studied using dedicated optical systems and measurement techniques based on visual psychophysics (flicker photometry), optoretinography, and electrophysiology. With the flicker photometry, the spectral two-photon sensitivity of individual photoreceptor types will be measured. We will compare the results with those obtained by optoretinography, which is based on optical tomography OCT and allows us to assess the extent to which photoreceptors are stimulated by light by analyzing changes occurring in the image of the retinal layers. We will also study visual evoked potentials and changes in the action potentials of retinal neurons induced by two-photon stimuli.

The project results will expand the knowledge of human visual perception, provide a better understanding of the mechanisms by which pulsed laser beams interact with the living eye, and allow us to collect the data necessary for further applications of the two-photon vision phenomenon.