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Clinical Application of fluorescence lifetime imaging at the eye

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Purpose: To determine the clinical value of measurements of fluorescence lifetime imaging (FLIM) in ophthalmology. **Methods:** A fluorescence lifetime mapper was used in this study, based on a modified Heidelberg Retina Angiograph. The fluorescence of endogenous fluorophores was excited by pico second lasers pulses at 446 nm. The time-resolved auto-fluorescence was detected by time-correlated single photon counting in a short-wavelength channel (490-560 nm) and a long-wavelength channel (560-700 nm). The pulse repetition rate was 80 MHz. The time of 12.5 ns between two pulses was divided in 1024 time channels, allowing a time resolution of 12.5 ps. The lateral resolution was 40µmx40µm. The decay of fluorescence after pulse excitation was approximated by tri-exponential model function. Histograms of lifetime t1, t2, and t3 as well as of amplitudes a1, a2, a3 in both spectral channels were calculated for global comparison. Local alterations were documented in images of these lifetime parameters. As several fluorophores form the integral decay of autofluorescence of the tissue, the tri-exponential fit is a mathematical correspondence only. Comparing with the anatomical structure, a certain relation exists between the short living component and the retinal pigment epithelium, the middle living component and the neuronal retina, and the longest lifetime is determined by connective tissue added by the residual fluorescence of the crystalline lens. The influence of single fluorophores can be estimated by external metabolic provocation by respiration of pure oxygen (FAD) or by internal provocation by retinal branch arterial occlusion (NADH). The time-resolved auto-fluorescence was measured in healthy subjects, dry/wet AMD, geographic atrophy, diabetics type II, arterial branch occlusion, and in macular foramina. **Results:** Considering histograms of lifetimes or of amplitudes, significant differences can be calculated between healthy subjects and dry AMD as well as diabetics. Most important are changes in lifetime t1 and t2 in the short-wavelength channel. In retinal branch artery occlusion, the lifetime t2 in the short-wavelength channel is elongated in non-supplied fields in comparison with supplied regions. As reason, a changed metabolic activity can be assumed caused by an increased contribution of glycolysis. Under these conditions, the concentration of protein-bound NADH is increased which has a long decay time of fluorescence. Alterations in the metabolism of the neuronal retina correspond to changes of t2 in the short wavelengths channel. The longest decay times are measured in Diabetes mellitus by the accumulation of advanced glycation end-products. Spots of elongated lifetime t2 in channel 1 can be found in Diabetes, comparable with t2 in non-supplied fields in arterial branch occlusion. In a simulation program, the influence of several different amplitudes and lifetimes on parameters of tri-exponential fit can be studied. This can be used for interpretation of measured changes in fluorescence decay parameters on human. This program can also be used for investigation of how many photons are required for discrimination of fluorophores depending on the difference of lifetimes and on the amplitudes. A principal solution for the determination of fluorescence decay in the layered structure of the eye will be given by an extension of the e-function for fitting the fluorescence decay. **Conclusion:** The time-resolved fluorescence measurement of endogenous fluorophores is a new method for functional diagnostics of metabolic changes in ophthalmology. The algorithm for approximation of fluorescence can be used as first step for functional tomography. The calculated distance between the fluorescent layers is the tomographic information and the functionality is determined by the fluorescence in the separated layers.