Title
Fluorescence lifetime imaging and correlation spectroscopy for studying GABA receptor activity in living tissue

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Authors
Yu, WM
Kuriashkina, L
Gratton, E

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Weiming Yu, Liana R Kuriashkina, and Enrico Gratton.

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Abstract

A multi-photon fluorescence microscope is used as a platform for studying receptor activity in a living brain slice, traditionally studied by electrophysiological measurements. gamma-Aminobutyric acid (GABA) subtype A receptor is believed to cause hyperpolarization of neuron membrane and inhibition of neuronal excitability via influx of extra-cellular chloride (Cl-). There has been great interest in knowing both the local chloride concentration of individual neuronal cells, as well as their GABA receptor activities [Wagner et al., Nature 387, 598-603, 1997]. We used scanning two-photon, time-resolved fluorescence microscopy to quantitatively determine local chloride concentration in a viable brain slice. A femto-second pulse laser was used in combination with a pulse picker to explore harmonics of the basic frequency to measure fluorescence lifetime in the microscope using the frequency domain method. Signal arising from different fluorescence lifetime components can be either enhanced or suppressed when using different harmonics. Local fluorescence intensity signals of the sample with and without chemical stimulation were recorded as a function of time, both at a single point, as well as at regions of interests. These signals not only reflect functional activities of the neuron receptor, but also contain valuable information such as local chloride concentration and its cellular diffusion coefficient.