

IMPROVEMENT OF SIGNAL TO NOISE RATIO IN FAST TWO PHOTON LASER SCANNING MICROSCOPY BY DOUBLING THE PULSE REPETITION RATE OF THE EXCITATION SOURCE

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1. MOTIVATION AND CONCEPT

The recording of fast events like Ca²⁺ transients in very small compartments like dendritic spines of neurons is challenged by the limited number of fluorescence photons available at high acquisition rates. Besides maximizing the photon collection efficacy of the system by means of non-descanned detection through high aperture objective and condenser lenses another way to gain more signal is the generation of more fluorescent photons at the same time. Here we follow this route by decreasing the duration of the idle, non fluorescent state of dyes in the time between two excitation pulses. Using a Coherent 5W Verdi-pumped Mira 900F-Ti:Sa-laser, which is a standard excitation source for two photon microscopy, the inter-pulse interval lasts for 13ns, whereas the fluorescent life time of most organic dyes is in a range of a few nanoseconds. We reduced the inter-pulse interval to 6.5ns and achieved thereby a higher excitation rate with unchanged peak power.

2. REALIZATION

The laser beam is divided by a polarizing beam splitter. One of the beams travels along a delay line whereas the other one reaches directly a second polarizing beam splitter where the two beamlets are unified again. The beam splitters and the delay line are tilted by 45° with respect to the plane of polarization of the original beam. Therefore virtually no power is lost by insertion of the system into the excitation light path. The relative delay of the two beams equals 6.5ns, i.e. the pulse repetition rate of the laser beam entering the microscope is effectively doubled to 152MHz with respect to the 76MHz delivered by Mira.

3. EXPERIMENTS

The following paradigms will be presented:

Measurements of fluorescence obtained following two photon absorption at different excitation intensities with 76MHz or 152MHz pulse repetition rate in solutions of Alexa 594 hydrazide and of Ca²⁺ indicators saturated with Ca²⁺; measurements of fluorescence intensities from fast xy-scans and line scans of fluorescent beads. These experiments address the question whether a reduction of the pulse interval results in an equivalent increase of the signal or a deterioration of fluorescence by saturation effects or photodestruction of the dyes. The fast scans show, to what extent the variance of the signal can be reduced by the increased pulse repetition rate. Finally, fast xy-scans and line scans of Ca²⁺ transients in dendritic spines of living neurons in organotypic brain slice cultures will test the applicability and usefulness of this technique for fast physiological measurements in biological samples.

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