Real time 3D nonlinear microscopy

B. Rozsa, E. S. Vizi
Institute for Experimental Medicine, Department for Pharmacology, P.O. Box 67, H-1450 Budapest, Hungary

G. Katona, A. Lukács
R & D Ultrafast Lasers Kft. P.O. Box 622, H-1539 Budapest, Hungary

Z. Várallyay, A. Sághy, L. Valenta, P. Maák
Budapest University of Technology and Economics, Budafoki út 8, H-1111 Budapest, Hungary

J. Fekete, Á. Bányász, R. Szipőcs*
Research Institute for Solid State Physics and Optics, P.O. Box 49, H-1525 Budapest, Hungary
szipoecs@sunserv.kfki.hu
**also with Institute for Experimental Medicine, Department for Pharmacology*

Abstract. We propose a nonlinear microscope scheme capable of real time, 3D investigation of the electric activity pattern of neural networks or signal summation rules of individual neurons in a 0.6 x 0.6 x 0.2 mm^3 volume with sub-micrometer spatial resolution.
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Introduction

Recent investigations in neurology pointed out the importance of nonlinear effects such as two-photon absorption and second-harmonic generation in high spatial resolution microscopy [1]. Dendritic integration at the cell level and arithmetics of neuronal signaling at the network level however show a need for real time measurements meanwhile keeping the advantages of nonlinear microscopy.

The problem of measuring within the signal transition time across neurons or neural networks has not been solved yet. The reason for this is the following: current nonlinear microscope systems obtain images by scanning a laser beam in 2D and the 3D volume can be reconstructed only after subsequent recording of hundreds of 2D images along the optical axis (z-axis). This procedure typically takes a few minutes which is several orders of magnitude longer than the signal transition time (< 1 ms) of a neuron. Looking at signal propagation in neurons the points to be investigated (such as dendritic spines) lie randomly distributed in 3D space. This requires the ability to carry out measurements on arbitrary points within a 3D volume well within the signal transition time.

In this paper we present a novel two-photon microscope scheme being capable of high speed measurement of signal transitions in neural networks or through single neurons in a 3D volume of approx. 0.6 x 0.6 x 0.2 mm^3.

Concept

As an initial step, a conventional 3D image is obtained by taking 2D scanning two-photon images of the biological sample. From this image the coordinates of those points (P_1, P_2 … P_n) are determined in the 3D volume that are to be investigated (see Fig. 1(a)). During the measurement of neural activity, only these points are sequentially addressed by a high speed acousto-optic (AO) switch combined with a fiber bundle (of n optical fibers) and a properly designed optical imaging system (see Fig. 1(b)).