Random access three-dimensional two-photon microscopy

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We propose a two-photon microscope scheme capable of real-time, three-dimensional investigation of the electric activity pattern of neural networks or signal summation rules of individual neurons in a 0.6 mm × 0.6 mm × 0.2 mm volume of the sample. The points of measurement are chosen according to a conventional scanning two-photon image, and they are addressed by separately adjustable optical fibers. This allows scanning at kilohertz repetition rates of as many as 100 data points. Submicrometer spatial resolution is maintained during the measurement similarly to conventional two-photon microscopy.

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1. Introduction

Two-photon microscopy is an effective tool for three-dimensional (3D) high-resolution imaging of biological specimens such as neurons *in vivo*.[1] The problem of measuring signals of neurons or neural networks within the signal transition time, however, has not been solved yet. Applications such as imaging of dendritic integration and arithmetics of neuronal signaling show the need for real-time measurements while keeping the advantages of two-photon or other types of nonlinear microscopy.[2,3]

The drawback of current two-photon microscope systems is that they obtain 3D images by scanning a laser beam in two dimensions. The 3D volume can be reconstructed only after subsequent recording of hundreds of 2D images along the optical axis (z axis). This procedure takes up to a few minutes, which is several orders of magnitude longer than the signal transition time (<1 ms) of neurons. Signal propagation needs to be investigated only in certain points (such as dendritic spines), which 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. Systems using piezofocusing to change the focus are able to acquire data from different focal planes to within hundreds of milliseconds but this is still longer than the signal transition time.

The idea of carrying out measurements only in certain points is realized in the following way. First a conventional 3D image is obtained by taking 2D scanning two-photon images of the sample. One has to determine the coordinates of the points (P_1, P_2, . . . , P_n) of interest from the reconstructed image [see Fig. 1(a)]. Next, the real-time measurement of neural activity is carried out. The selected points are sequentially addressed by a high-speed acousto-optic (AO) deflector combined with a fiber bundle (of n optical fibers) and a properly designed optical imaging system [see Fig. 1(b)] by aligning the fiber ends by micropositioning mechanics[4] and imaging them onto the selected points of the sample.

We present a novel two-photon microscope scheme that is capable of high-speed measurement of signal transitions in neural networks or through single neurons in a 3D volume of approximately 0.6 mm × 0.6 mm × 0.2 mm. In Section 2 we describe the ar-

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