HIGH-SPEED, HIGH-FREQUENCY SCANNING OF USER-DEFINED 3D REGIONS OF INTEREST USING TWO-PHOTON MICROSCOPY

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The technique developed in our group allows scanning user-defined trajectories (trajectory mode) or acquiring consecutive two-dimensional images (image mode) in three dimensional samples at maximal measuring speed.

In contrast to previously presented methods [1, 2] our technique does not use predefined scan trajectories or scans restricted in the axial dimension. Rather our trajectory mode connects user-defined points in space not limited by number and scans them either in order of input or in the fastest calculated way. This technique was shown to work in the GCaMP-stained antennal lobe of drosophila. It will be used to identify olfactory glomeruli matching specific odors and to visualize the time-resolved reactions of the glomeruli distributed in 3D. The image mode allows scanning user-defined two-dimensional images lying in different depths repeatedly with high frequency. This was used to image a group of neurons within a chick dorsal root ganglion bulk-stained with Fluo-3 upon stimulation of a neighboring neuron. A rectangular region of interest was fit into each neuron in order to increase the signal to noise ratio compared to a single line going through the cell. 2D scans were repeatedly imaged to record the temporal behavior of the calcium signal.

Achievable frequencies (typically between 5 and 50 Hz) depend on number and location of the trajectory points or 2D scans respectively.

A home-built upright two-photon laser scanning microscope was used. It was equipped with a digitally controlled voice-coil z-drive for fast and precise axial movements within a range of several millimeters, and an also digitally controlled laser-scanhead (Yanus, TILL Photonics) for simultaneously scanning the focus in x and y. The user interface for the construction of the individual scans was developed in LabVIEW and implemented into “Colibri”, a freely available software tool also developed at the BIZ [3].

