

# FAST 3D 2-PHOTON CALCIUM IMAGING IN THE ANTENNAL LOBE OF *DROSOPHILA*

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The antennal lobe (AL) of the fruit fly (*Drosophila melanogaster*) is a 3-dimensional structure consisting of a number of well-defined zones of dense synaptic connectivity called glomeruli. The spatio-temporal activity pattern in these glomeruli defines the neural code for olfactory stimuli in the fly's environment. In combination with the genetic tools available, this makes *Drosophila* an ideal system for identifying generic coding mechanisms in neural systems. When a calcium sensitive reporter protein (e.g. GCaMP) is transgenically expressed in defined neuronal populations (e.g. in receptor neurons or in interneurons), optical imaging allows the recording of odor-evoked spatio-temporal activity patterns across glomeruli. These techniques have been successfully implemented using wide-field microscopy [1,2].

However, wide-field microscopy techniques only allow for simultaneous activity measurements in a 2-dimensional projection of the structure and therefore only a subset of glomeruli can be evaluated. Therefore, here we use a method pioneered by [3] in which the focus point of a 2-photon microscope travels through a volume at a high speed along a predefined path. We have adapted this 3D-line-scan technique for use in insect preparations. This allows for the quasi-simultaneous measurement of whole brain areas in vivo.

In a second step, we have developed a technique to reconstruct the volume of the AL from the line-scan. We employ covariance-free PCA strategies for efficiently reducing the dimensionality of the large data volumes, and subsequently perform source separation using an Independent Component Analysis (ICA) approach. The framework is an extension of our previous work on the analysis of 2D recordings in the antennal lobe of honeybees [4]. Our method is able to detect the relevant glomerular signals in noisy recordings.

We show preliminary data from the whole AL of living *Drosophila* when stimulated with a range of different odors.

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