4D in-vivo morpho-functional imaging of the honeybee brain

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We report on the set-up of a two-photon microscopy platform for in-vivo insect neuroimaging. Resonant piezo scanning mirrors allow for time-resolved high resolution planar imaging or 1D random shape line scans. A piezo-driven objective motor permits fast 3D volume scans and transversal plane scans.

The western honeybee Apis mellifera was chosen as a model to study olfactory information coding and memory-formation-related plasticity in a medium-sized brain of about a million neurons with extraordinary learning performance. We started focusing on the primary olfactory neuropil in the honeybee brain, the antennal lobes (AL). The set-up allows obtaining both morphology data of the full AL due to the technique’s high penetration depth, as well as time-resolved in-vivo calcium imaging of its neuronal activity which was so far limited to the surface of the AL [1].

The morphological data was used to precisely measure the glomerular volume on both sides of the brain, investigating the question of a possible volumetric lateralization [2]. Functional calcium imaging allowed recording of the odour stimuli response maps of the antennal lobe’s functional units, the Glomeruli. These maps, representing the spatial odour code, can now be extended to the classes of sub-surface Glomeruli T2-T4, so far inaccessible to functional imaging. Thanks to the high acquisition rate of the instrument, we present first indications of a temporal dimension of the odour code, which manifests itself in odour-specific response delays. Finally, the reduced photo damage of two-photon microscopy allows for long time in-vivo experiments which may permit real time observation of neuroplastic changes of the olfactory processing network and thus direct monitoring of memory formation.