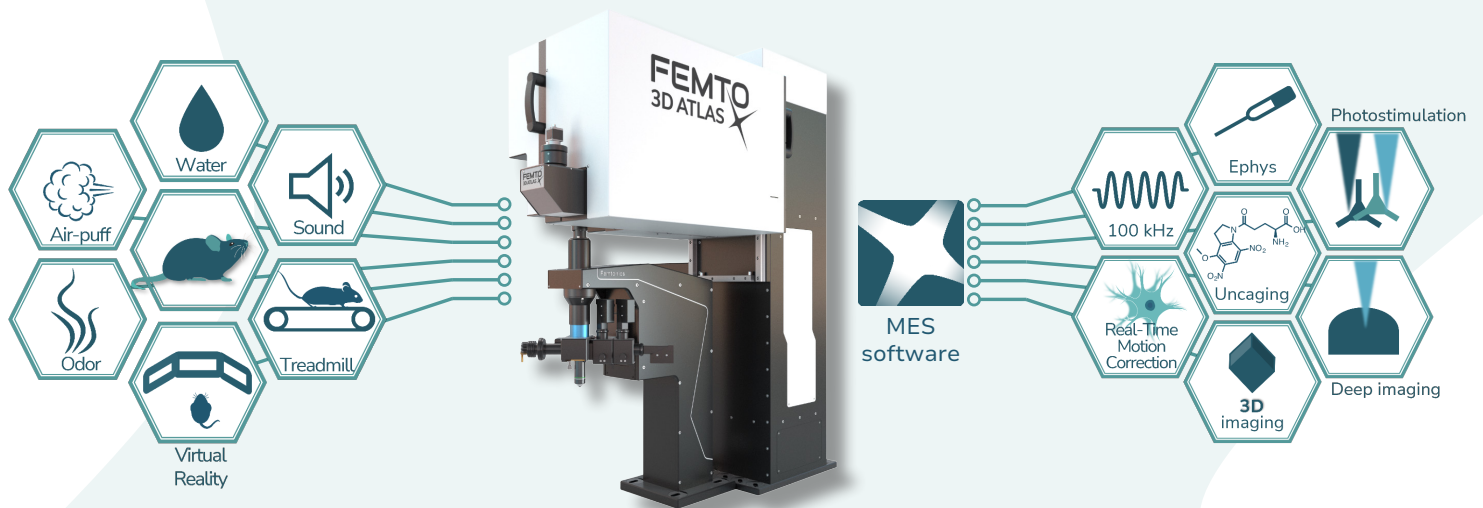


BEHAVIORAL EXPERIMENTS

FEMTO 3D ATLAS

Why choose the Atlas for behavioral experiments?

- 2p (two-photon) imaging is crucial for understanding neural mechanisms underlying behavior.
- Enables precise observation of neuronal activity at a **100 kHz speed** in individual cells within live animals.
- Provides **high spatiotemporal** resolution insights into neural circuits driving complex behaviors.
- Allows manipulation of neural activity to establish causal links between neural circuits and behavior, with **photostimulation and uncaging** options.
- With **real-time** motion correction aiding the measurements, the signal-to-noise ratio can be magnitudes higher in vivo.
- Compatible with state-of-the-art **VR technologies**.



How can VR enhance behavioral experiments?

VR possibilities - provided by BrainVisionCenter

- **Natural behavior** require experiments replicating real-world conditions.
- **Control and stability** during behavioral experiments are ensured by combining virtual reality (VR) with head-fixed recordings.
- **Immersion** in interactive virtual environments is convincingly created for animals using VR.
- **Accelerated learning** occurs as animals perceive virtual environments as reality.



A schematic VR landscape for immersive behavioral studies


✦ Is the Atlas capable of experiments on a wide range of species?

➤ 3D AO chessboard imaging during random foraging in MICE

TWO-PHOTON IMAGING

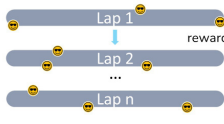
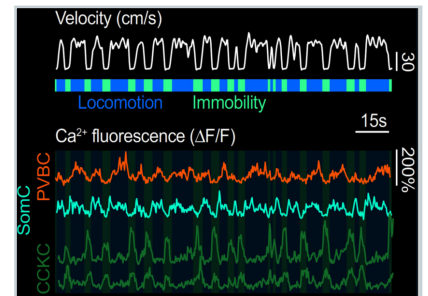
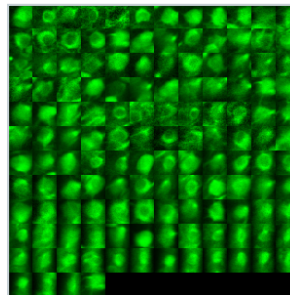
Chessboard scanning by FEMTO3D Atlas:

- 200+ interneuron
- @ 4-5 Hz



BEHAVIOR

Schematic of the random foraging task





Water-restricted mice were trained to run on a tactile fabric belt and lick for water rewards. Velocity was recorded, and hippocampal interneurons were imaged using the Chessboard scanning mode of the FEMTO3D Atlas. The figure shows over 200 interneurons imaged during random foraging, with locomotion states linked to Ca²⁺ activity in specific hippocampal interneurons.

Learn more: Geiller et al. *Neuron* (2020)

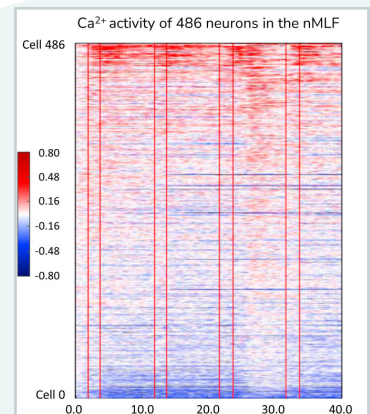
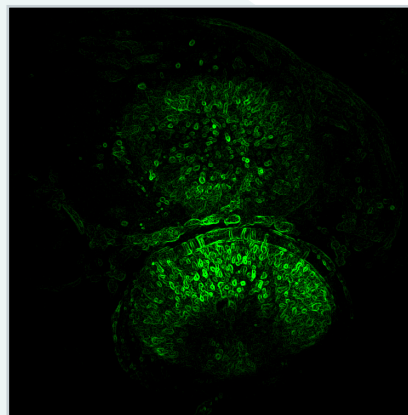
➤ Simultaneous 3D imaging, photostimulation and behavior recording in ZEBRAFISH

TWO-PHOTON IMAGING AND PHOTOSTIMULATION of the nucleus of the medial longitudinal fascicle (nMLF)



BEHAVIOR TRACKING

Tail bending




Dal Maschio et al. developed a method combining two-photon imaging, 3D photostimulation, and behavioral tracking in zebrafish larvae. Tail movements were triggered by photostimulation, while neuronal activity and behavior were recorded. The left panel shows the experimental setup, and the right panel displays calcium activity from 486 neurons and tail kinematics in response to stimulation. Red lines indicate the start and end of photostimulation.

Learn more: dal Maschio et al. *Neuron* (2017)

➤ 3D imaging during short term memory testing in DROSOPHILA

TWO-PHOTON IMAGING

+ Odor stimulation




5x

1.5 s 30 s 1.5 s 30 s

OCT break MCH break

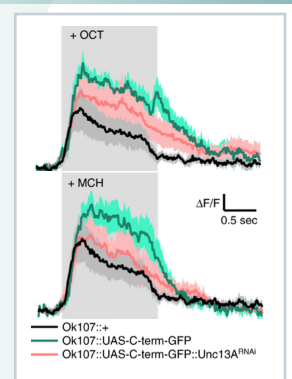
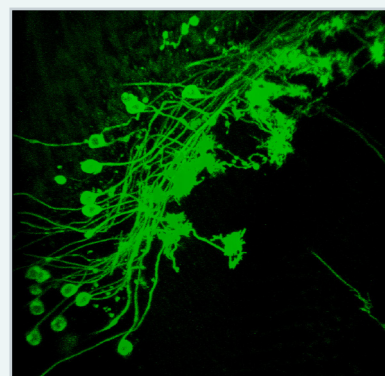
BEHAVIOR:

Aversive olfactory conditioning:



3-Octanol (OCT) + electrical shock

Methylcyclohexanol (MCH)



Böhme et al. used two-photon imaging to record calcium responses during a short-term memory test in *Drosophila*. Flies were trained by pairing an odor with an electric shock. Calcium activity in neurons was evoked by odors during the imaging and behavioral experiment. The right panel shows averaged odor responses in control and mutant flies, with five responses averaged per odor per animal.

Learn more: Böhme et al. *Nat Commun* (2019)



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