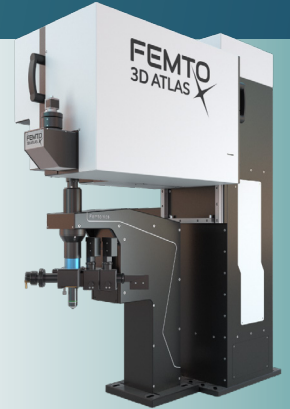


VARIOUS ACOUSTO-OPTIC TECHNIQUES

FEMTO
3D ATLAS



FAST 3D FOCUSING ON DEEP STRUCTURES POSSIBLE?

GRIN lens imaging

- GRIN lens imaging works by inserting a gradient lens, **enabling focusing in deeper regions** of the brain.
- FEMTO3D Atlas microscopes can combine **acousto-optics and GRIN lenses** for precise and fast (100 kHz) 3D in vivo imaging through the lens, even during behavior. No further movement of the lens is required.
- GRIN lenses **mitigate photodamage** risks in deep brain structure imaging, and allow imaging in regions up to 8000 μm below surface.

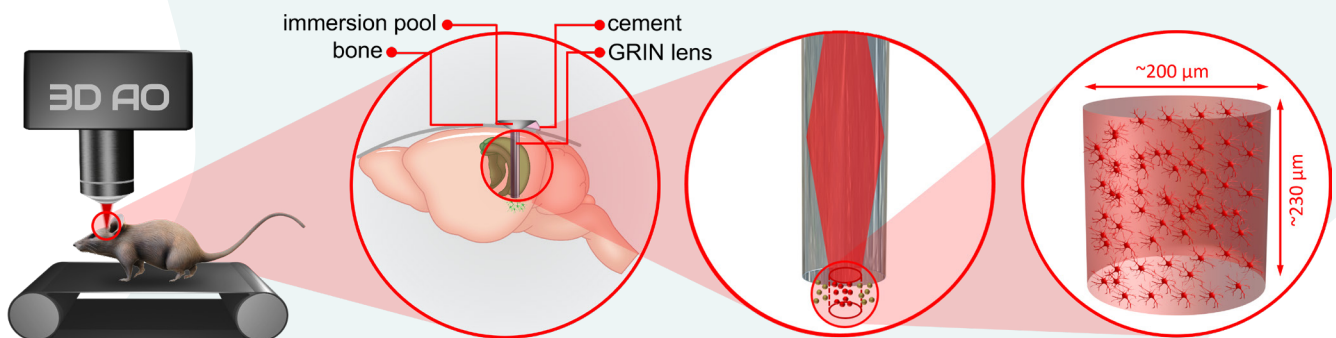


Figure 1: Bird's-eye view of the processes of GRIN lens imaging. The lens is inserted at the preferred location under which the 3D space (200*200*230 μm) is freely observable and measurable.

FLIM AND VOLTAGE IMAGING COMBINED?

Simultaneous FLIM and 2p AO voltage measurements

- **Dual readout:** Captures both metabolism (FLIM) and excitability (voltage).
- **Direct linkage:** Correlates energy use with electrical activity.
- **Cleaner signals:** FLIM resists intensity artifacts; voltage gives speed.
- **Broader insight:** Reveals coupled metabolic–electrical dynamics in health and disease.

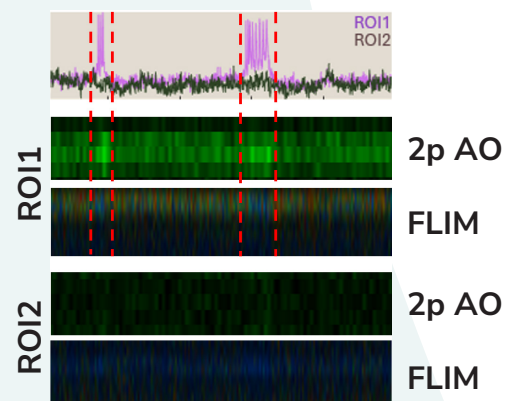


Figure 2: Simultaneous FLIM and voltage measurement in vivo. ROI1 shows signals with both imaging techniques simultaneously, while ROI2 stays silent together.

IMAGING IN INSECTS?

✦ **Drosophila imaging with the FEMTO3D Atlas**

- AO offers **high-resolution images** with minimal photodamage, from any angle without any difficult surgical intervention using **high-speed arbitrary frame scanning**,
- Rapid scanning enables **real-time visualization of neural activity** with voltage and calcium indicators, even simultaneously, from even both hemispheres.
- Ideal for comprehensive 3D imaging and defining **complex regions** of interest, even in long-term (chronical) studies, with **real-time motion correction**.

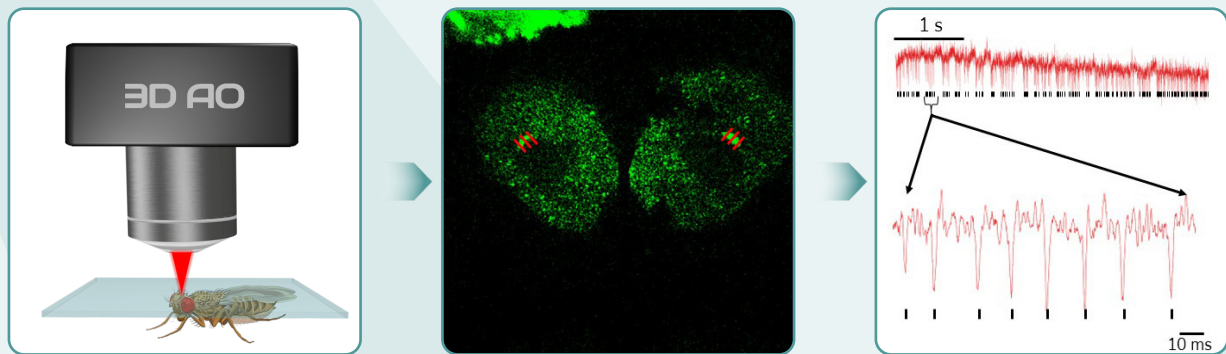


Figure 3: Voltage Imaging of Drosophila; including a Schematics of Drosophila 2p imaging, ASAP labelled Drosophila neurons. Red lines over the somata are representing the line scan locations and the ASAP signal from the labelled neuron recorded with 6675.57 Hz.

LONG TERM IMAGING SUSTAINABLE WITH THE FEMTO3D ATLAS?

✦ **Time-lapse imaging**

- Time-lapse imaging is a powerful technique in developmental neuroscience, capturing **neural development over extended periods**.
- Combining acousto-optic two-photon microscopy's **outstanding spatial resolution** with time-lapse imaging's temporal dimension offers unprecedented insights.
- Real-time motion correction helps providing a window into cellular and molecular events shaping the developing brain by **stabilizing the images** throughout the imaging process.
- Z-Stack measurements have indefinite length, with specialized software for **adjustable protocols**.

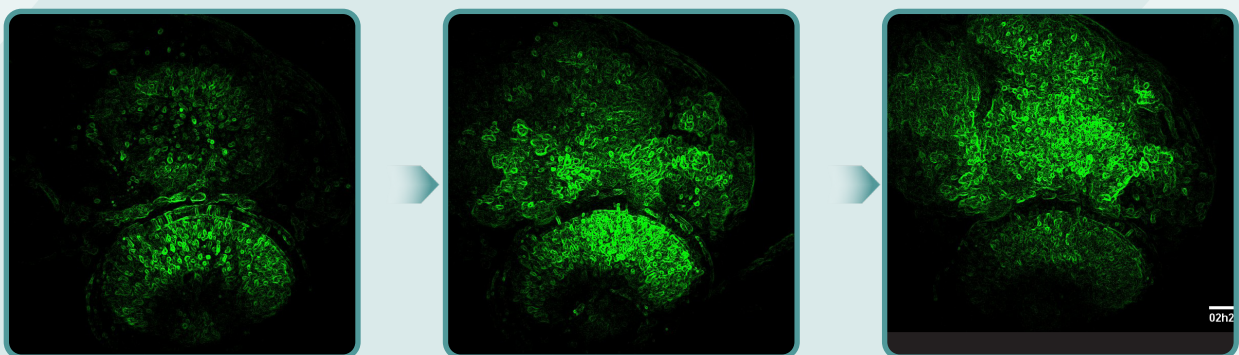


Figure 4: Time-lapse imaging of zygotic Zebra Danio eye with example images taken at timepoints 0, 60, and 150 mins.



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