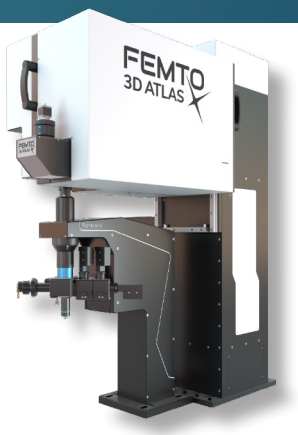
PHOTOSTIMULATION AND UNCAGING WITH ACOUSTO-OPTICS

FEMTO 3D ATLAS



What optogenetic tools are available with the ATLAS?

- Photostimulation techniques **precisely activate cells**, crucial for studying synaptic plasticity, learning, and memory.
- The FEMTO3D Atlas **Dichro** allows simultaneous optogenetic stimulation, uncaging, and calcium imaging using a second laser.
- **Uncaging** involves **releasing bioactive molecules** from caged compounds upon light exposure.
- This setup enables **cellular activity manipulation** through both optogenetic techniques and chemical uncaging.

Why use 3D ATLAS for photostimulation?

Precise 3D Targeting:

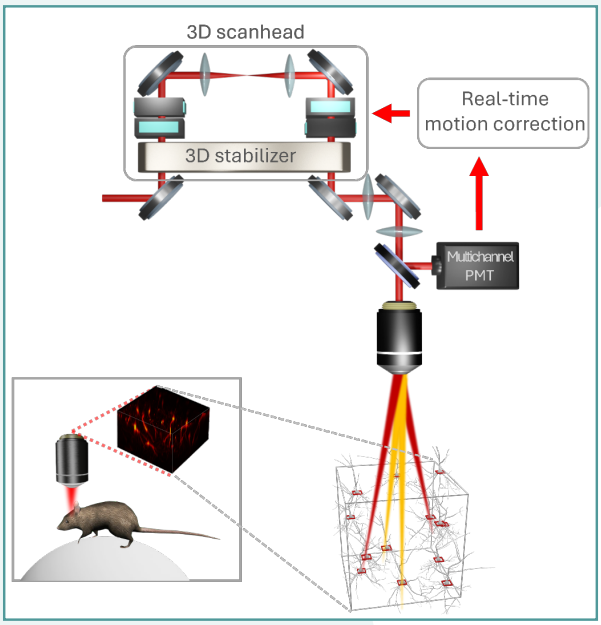
Users can choose 3D scanning patterns to accurately stimulate sparsely distributed individual cells or dendritic processes within a large volume.

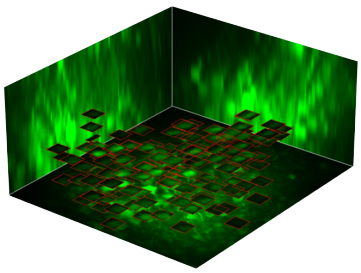
Simultaneous Recording, Stimulation and Motion Correction:

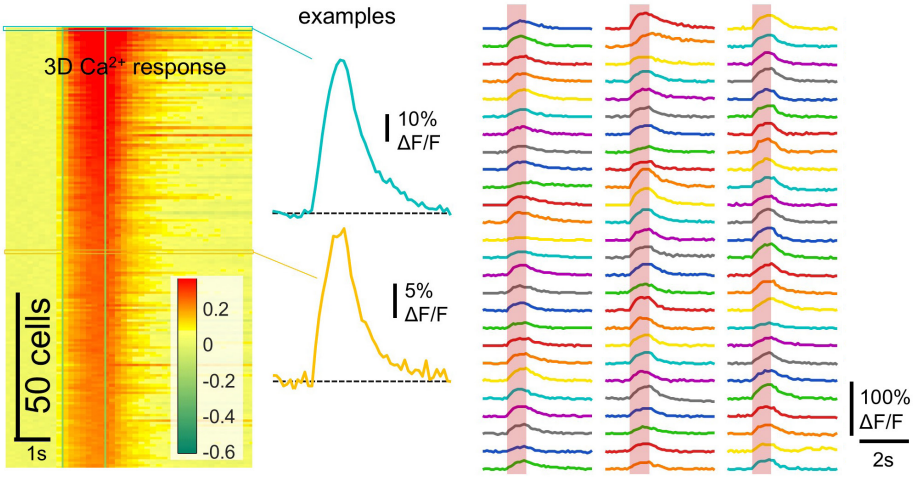
Swift alternation between two laser lines enables near-simultaneous recording of activity and Real-time motion correction ensures precise stimulation.

Cost-Effective and Easy Alignment:

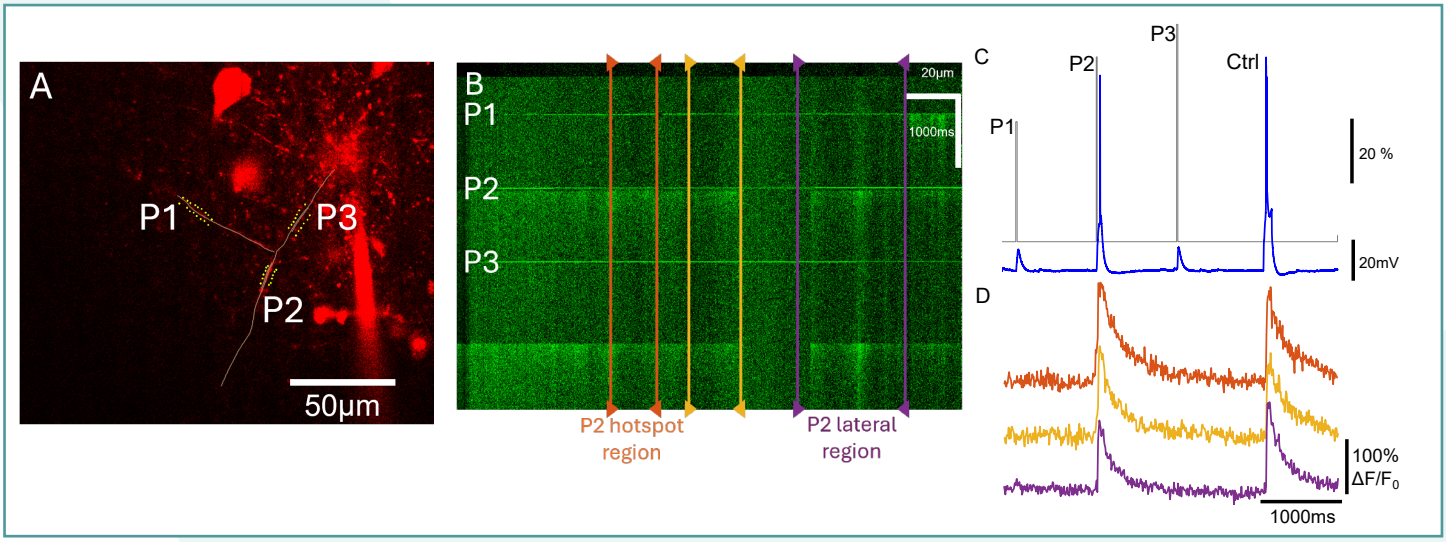
A single AO scan-head is used for both imaging and photostimulation, making the setup cost-effective and easy to align.







What are the benefits of uncaging?



Complex patterns 3D acousto optics uncaging (A) Single plane fluorescence image patched cell filled with ROD-2. P1,P2,P3 indicate the uncaging locations in different z level, white circle shows the somata. Grey line the imaging line during uncaging.(B) Calcium map of an uncaging stimulation. P1,P2,P3 are uncaging locations, the last stim is a control somatically evoked action potential. Colored lines are ROIs from the imaging line. (C) Uncaging protocol (grey) and simultaneously recorded electrophysiological recordings (blue). (D) Integrated fluorescence signals from panel B. Note, during P2 stim huge calcium signal appeared both in hot stop and lateral region. Ideal experiment design for complex pharmalogical studies.

- **Controlled Release:**
  Uncaging releases bioactive molecules from caged compounds upon light exposure, combining molecular chemistry and neuroscience.
- **Precision in Research:**
  Researchers can liberate neurotransmitters or ions with precise temporal and spatial control to explore cellular signaling pathways and synaptic function.
- **Insight into Molecular Interactions:**
  This technique provides invaluable insights into fundamental molecular interactions within cells.

How do we support uncaging measurements?

- **Simultaneous use of two laser beams** with different wavelengths.
- The two precisely aligned laser beams are stabilized by the integrated **beam stabilization unit** (4DBCU).
- Stimulation is possible with a **millisecond temporal resolution** at 100 or more locations.
- Ability to switch between the two laser beams every ~30 µs, with a **transition as short as ~10 µs**.
- Novel and **better uncaging compounds** (DNI-Glu) developed in-house by Femtochemistry.

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