3D PHOTOSTIMULATION

FEMTO 3D ATLAS Dichro

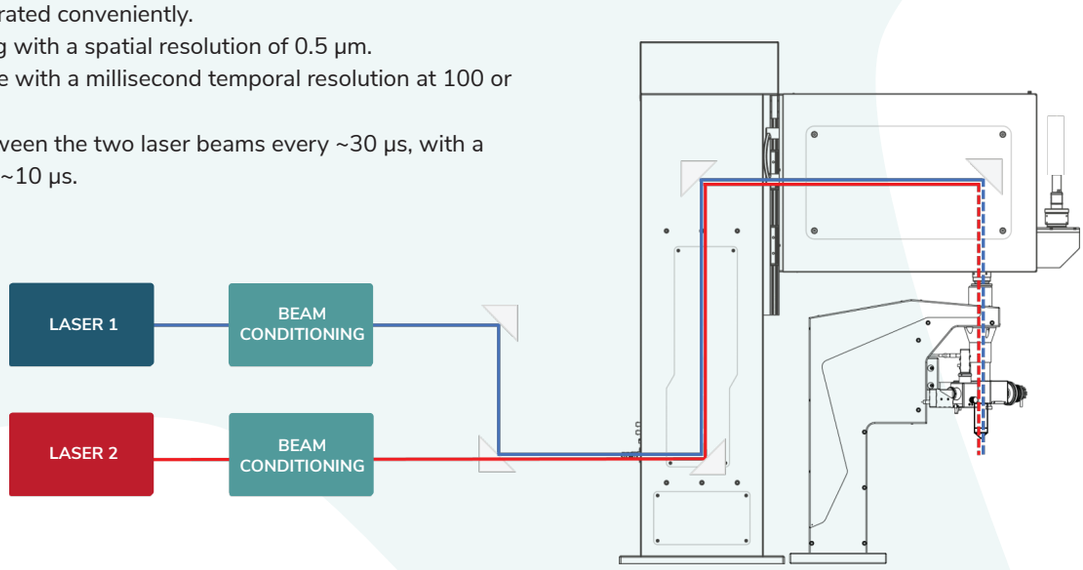
THE NEW FEATURE OF OUR ACOUSTO-OPTIC SCANNER-BASED MICROSCOPE FAMILY

Photostimulation techniques allow activating cells in a selective and precise manner, which makes them advantageous for various biological applications, from studies of synaptic plasticity to experiments investigating learning and memory *in vivo*. To the FEMTO3D Atlas Dichro extension a second laser can be coupled enabling users to perform **optogenetic stimulation** or **uncaging** with calcium imaging simultaneously, even in different cell populations.

SIMULTANEOUS 3D PHOTOSTIMULATION AND 3D IMAGING

Using the 3D random-access excitation method of FEMTO3D Atlas Dichro, scanning methods of the Femtonics AO technology are available with flexible parameters for photostimulation. Depending on the selected scanning pattern, users can stimulate sparsely distributed individual cells or dendritic processes in a large volume with high precision. By rapidly switching between the two laser lines, activity can be recorded near-simultaneously with photostimulation. Using a single AO scan-head for imaging and photostimulation makes the feature cost-effective and easy to align.

- Simultaneous use of two laser beams with different wavelengths (for example, 750–970 nm and 1030–1050 nm).
- The two precisely aligned laser beams are stabilized by the integrated beam stabilization unit.
- The improved acousto-optical crystals provide a high transmittance efficiency, even at longer wavelengths.
- Patterns can be generated conveniently.
- Independent scanning with a spatial resolution of 0.5 µm.
- 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.



THINKING AHEAD FEMTONICS MICROSCOPY

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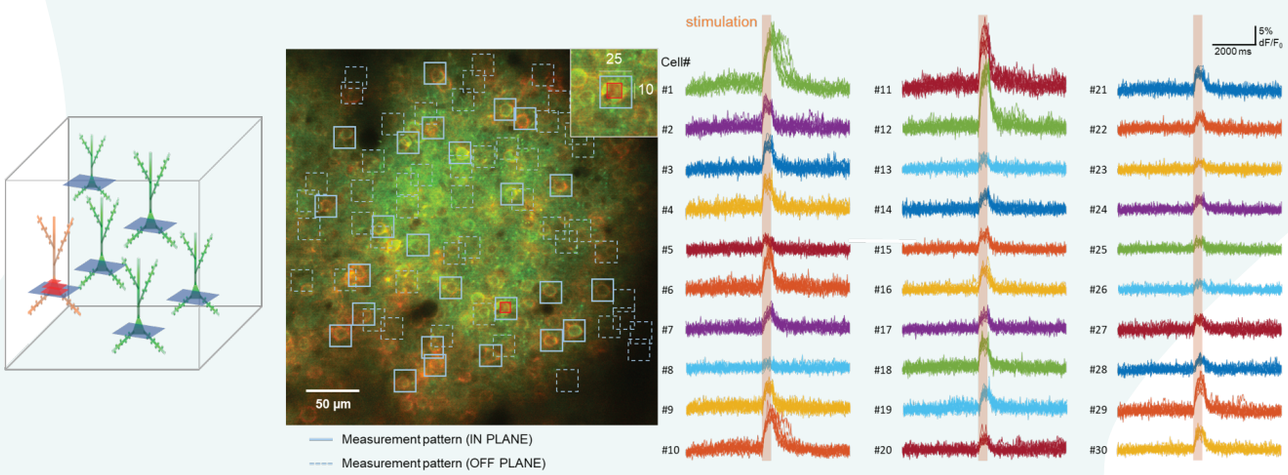
APPLICATION EXAMPLE

Photostimulation can be interlaced with imaging by selecting a scan area covering one or more somata situated anywhere in the 3D field of view. Stimulation can be performed by the 3D chessboard scanning mode[1] which is an advanced experimental approach, that extends 3D random-access point scanning into small local planes. Distributing the stimulation energy homogenously onto the somata of the selected cells allows maximum stimulation efficiency, thereby minimizing the possibility of photodamage to the targeted cells. The laser beam used for photostimulation excites the channelrhodopsin expressed in the cells, eliciting a controlled burst of action potentials. For calcium imaging a second scanning pattern with larger regions can be selected, enveloping cell bodies with the surrounding areas, in which the evoked activity can be followed even during sample movements. Timing of the photostimulation and the imaging can be precisely controlled by various protocols from the software GUI. This way the connectivity of the network elements can be examined, and correlated with the network function.



2. Figure: ChrimsonR-mRuby2 and GCaMP6f expressing cells were stimulated and imaged by the 3D chessboard scanning method in the cortex of a mouse in vivo. Red square: stimulation of a cell at 10 x 10 µm, green squares: imaging of cells at 25 x 25 µm/cell in the presented plane (continuous line) and at other Z depths (dotted line). Stimulation was performed with 10 x 1 ms pulses (10 Hz repetition rate) for higher efficiency, imaging was performed with 12 Hz.

[1]see the *3D anti-motion technology* whitepaper for details

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