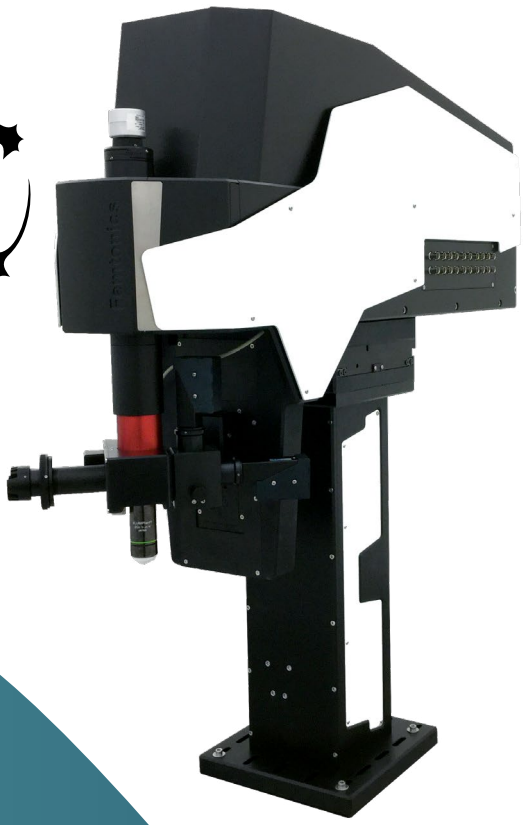
SIMULTANEOUS IMAGING AND PHOTOSTIMULATION

FEMTO SMART Dual



THINKING AHEAD
FEMTONICS MICROSCOPY

SIMULTANEOUS IMAGING AND PHOTOSTIMULATION

DUAL LIGHT PATH FOR HIGH-RESOLUTION PHOTOSTIMULATION

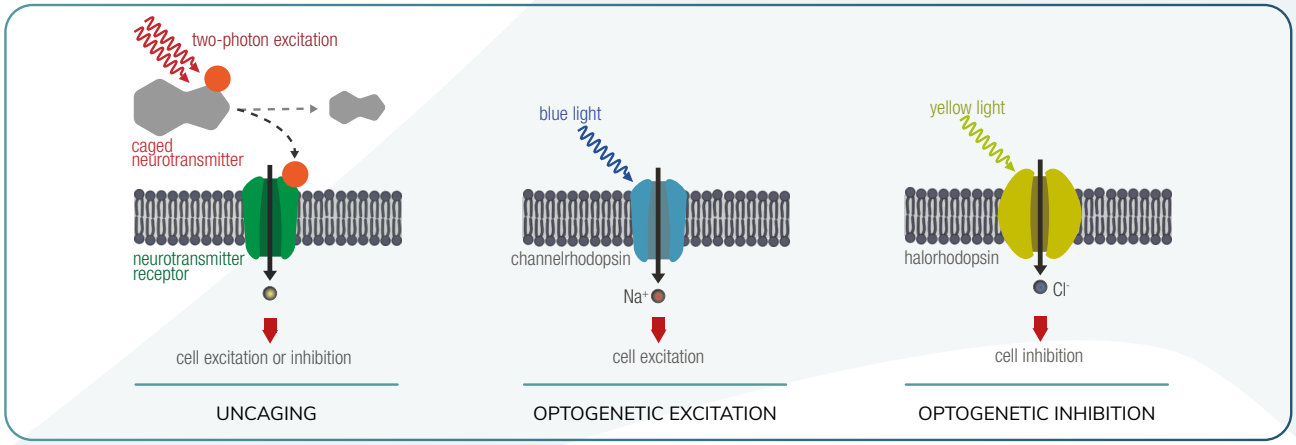
Multiphoton microscopy is the method of choice for accessing tissue function at cellular and subcellular scales *in vivo*. In contrast to single-photon imaging, multiphoton excitation can penetrate deeper into the tissue, reaching the depth of 1 mm. The FEMTOSmart Dual multiphoton microscopy platform is a modular yet affordable solution with multichannel laser excitation and multichannel fluorescence detection, suitable for imaging a wide range of chromophores. The foundation of Femtonics microscopes is their state-of-the-art optics and decade-long continuous user interface development. FEMTOSmart Dual microscopes integrate two independent light paths into one system thereby offering simultaneous imaging and photostimulation at high spatial and temporal resolution.

ALL-OPTICAL BIDIRECTIONAL TISSUE ACCESS VIA UNCAGING ACTIVE COMPOUNDS

Light can be used to modulate tissue function at high spatio-temporal precision. The first era of light-controlled modulation of tissue function used a single-photon ultraviolet light source to uncage biologically active compounds. During the last decades of the 20th century a wide range of caged compounds were designed and used *in vitro* in uncaging experiments. In 2000, MNI-Glu, the first compound suitable for two-photon uncaging was introduced (Matsuzaki et al., 2000) which opened the way for precise control of glutamate levels down to the level of dendritic spines, *in vivo*. Today, uncaging represents a form of light-controlled pharmacology where the spatio-temporal precision of the focused light beam can constrain the action of the compound in space and dose.

SIMULTANEOUS OPTOGENETIC ACTIVATION AND FLUORESCENCE IMAGING

Recently, a new way of using light to access neuronal activity revolutionized neuroscience. Optogenetics relies on genetically-targeted expression of light-sensitive actuator proteins that can shift the membrane potential of cells hit by light (Deisseroth, 2015). Optogenetic actuator proteins can shift the membrane potential to depolarization or hyperpolarization. Several actuators have optimized variants with improved efficiency or with red-shifted excitation wavelengths. Both uncaging and optogenetics surpass classical activity modulation techniques because light can simultaneously report activity from many cells and modulate the activity of a multitude of cells.



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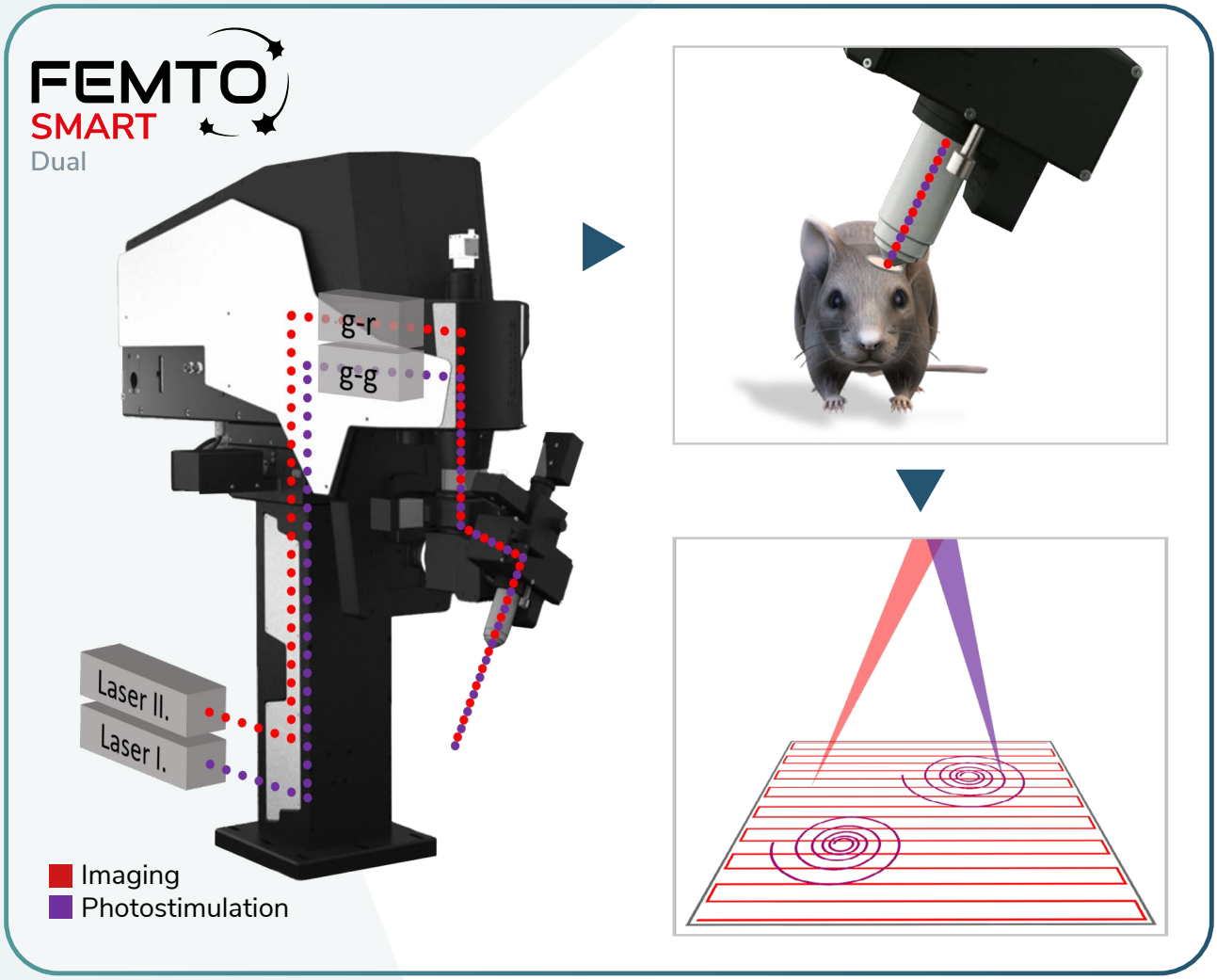
FEMTOSmart Dual microscopes that have a second photostimulation light path are an affordable choice to realize your uncaging or optogenetics experiments. For all-optical access to neuronal function, two independent laser sources and their light paths are required that we offer as a new system, the FEMTOSmart Dual, or as an upgrade to existing FEMTOSmart systems.

TECHNICAL OVERVIEW OF THE DUAL LIGHT PATH

FEMTOSmart Dual systems use a dichroic mirror to combine the laser beam reflected by the conventional galvo-galvo scan head with the second laser beam reflected by the galvo-reso scan head. Precisely aligned beams can be used to perform simultaneous imaging and photostimulation at the same locations in the specimen. In most experimental protocols, the galvo-galvo scan head is used to sprint across target locations that need to be photoactivated while the galvo-reso scan head scans a large rectangular area to record activity of many cells, monitoring the effect of photostimulation over the whole field of view. The galvo-galvo scan head allows the coverage of target locations by patterns such as points, lines or spirals. The choice of pattern used for photostimulation is related to the nature of the target: point-like spots are suitable to target dendritic spines, scan-lines can efficiently cover neuronal arborization whereas spirals are most suited for delivering strong stimulation to cell bodies.

Light path configurations include uncaging- or optogenetics-optimized selections of mirrors and filters or any other configuration that matches the experimental requirements of our customers.



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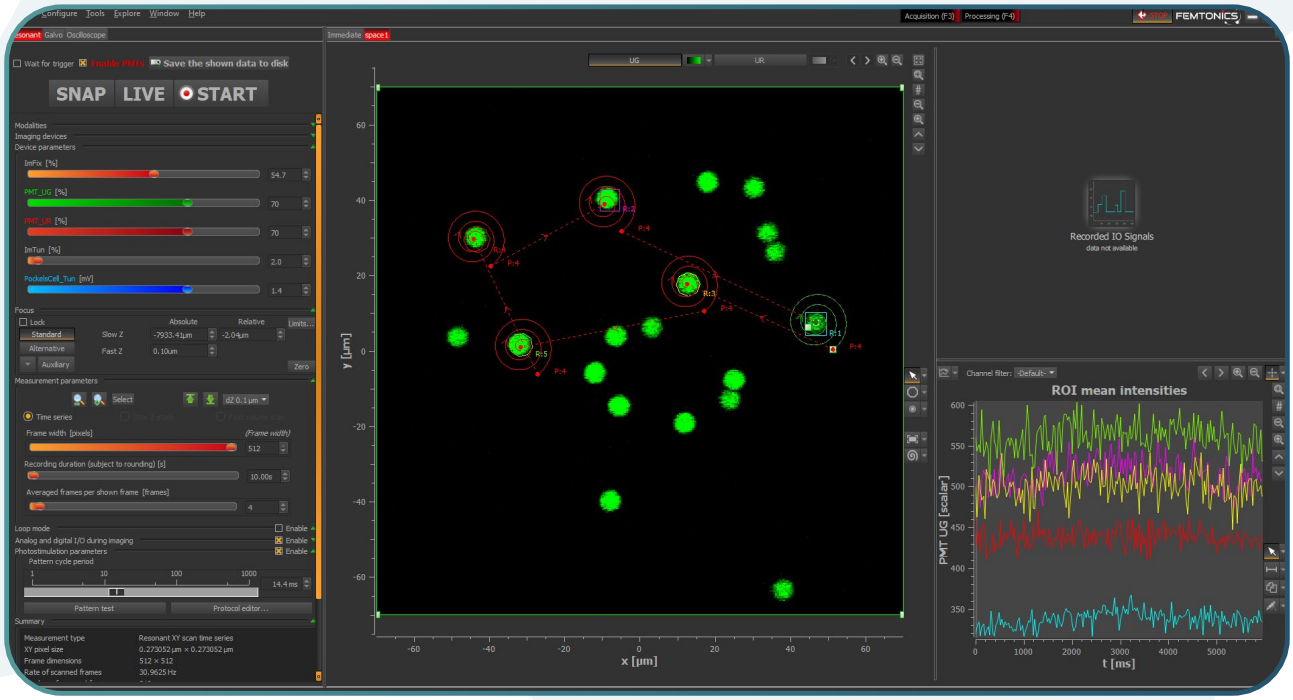
ADVANTAGES OF SIMULTANEOUS IMAGING AND PHOTOSTIMULATION WITH FEMTOSMART DUAL

- **Truly simultaneous:** optical activity readout and modulation is implemented with two independent light paths. One laser beam is routed to the galvo-galvo scan head that employs conventional galvanometric mirrors to scan the beam along the two perpendicular axes of the focal plane. This scan head offers flexible positioning of the laser beam thereby scanning trajectories can cover many locations where cells or smaller features are to be photoactivated. In the other light path, the resonant mirror of the galvo-reso scan head allows fast, up to 31 Hz imaging of the full field of view.
- **Flexible configuration:** laser sources can be matched to optical light path parameters that optimally serve optogenetic, uncaging or other applications.
- **Integration with external devices:** the FEMTOSmart platform has been integrated into a wide array of experimental paradigms, working with electrophysiology setups, behavioral platforms, virtual reality setups and many others.
- **Easy to use:** motorized hardware, intuitive and user-friendly graphical user interface, streamlined data processing.

SOFTWARE SOLUTION FOR CONFIGURING, VISUALIZING AND PROCESSING PHOTOSTIMULATION EXPERIMENTS

The FEMTOSmart Dual software enables the user to select stimulus patterns covering regions of interest distributed across the field of view. Photostimulation parameters can be set in a streamlined interface to create a complete protocol of your experiment.



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