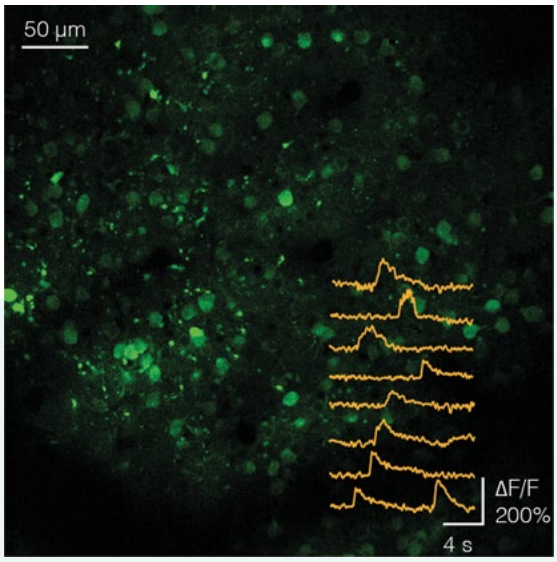
THREE-PHOTON EXCITATION

THE THREE-PHOTON (3P) MICROSCOPY allows noninvasive structural and functional imaging by making cells visible in deep tissues with high spatial resolution and better contrast compared to two-photon excitation.

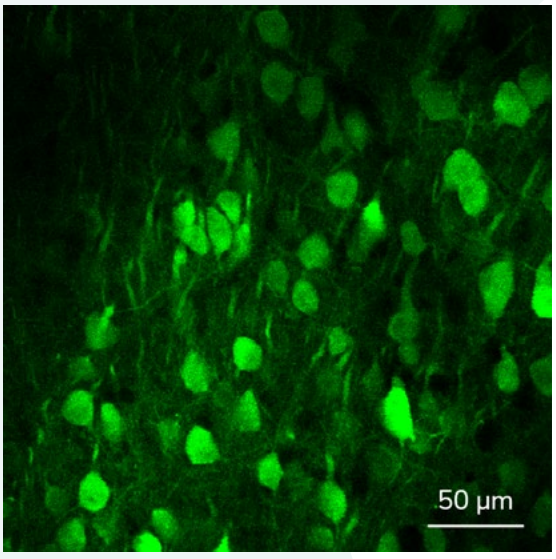
3P excitation is performed at an excitation wavelength range of 1,200-1,700 nm provided by an amplified laser system. Longer excitation wavelengths scatter less in biological tissues, which extends penetration depth, reduces out-of-focus excitation, and increases the signal-to-noise-ratio. The optical design required for 3P excitation permits a higher axial resolution than that of two-photon microscopy. The spectral window enables the three-photon excitation of a variety of fluorophores, such as the current generations of protein-based genetically encoded calcium indicators (e.g. GCaMP6) and the repetition rate of the laser source is adequate for imaging Ca^{2+} transients produced from neural activity.



Fluorescent activity measured on several somata from the V1 region of GCaMP labeled mouse

FEATURES

- excitation wavelength range: 1,200-1,700 nm
- functional and structural imaging through the whole cortical depth; advantageous in strongly scattering samples
- XY resolution is diffraction-limited (less than 1 µm, wavelength-dependent), Z resolution ~ 5-10 µm (sample and wavelength-dependent)
- excites fluorophores with the 3P excitation phenomenon: 3P wavelength range (1,200-1,700 nm) corresponds to the regular (1P) excitation wavelength range of fluorophores ranging from blue to red
- less tissue damage compared to 2P excitation



Spontaneous neuronal activity in the GCaMP6f-labeled visual cortex of a mouse. The cells were excited using a 1300 / 1400 nm laser wavelength.

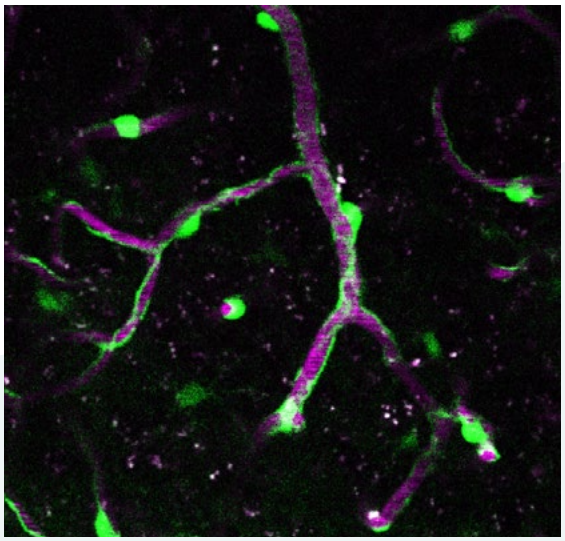
THINKING AHEAD
FEMTONICS
MICROSCOPY

Femtonics Ltd. HQ
info@femtonics.eu | www.femtonics.eu

Femtonics Inc. USA
usa@femtonics.us

BENEFITS

- functional and structural imaging through the whole cortical depth
- provides an advantage in strongly scattering sample
- Third Harmonic Generation option available



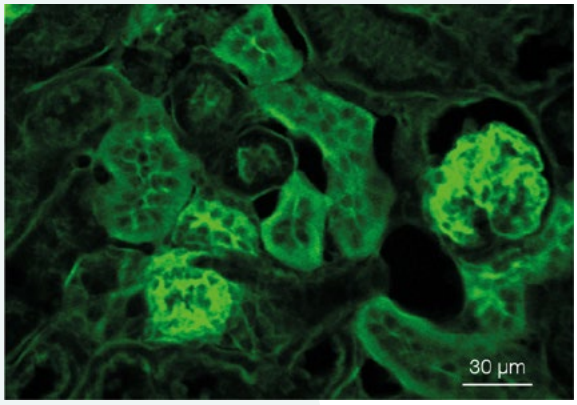
3P anatomical imaging: image of a mouse cortex from a 1000 µm depth. Purple: blood vessels, green: GFP labelled pericytes.

3P UPGRADE CONSISTS OF

- a pump laser and a Noncollinear Optical Parametric Amplifier (NOPA)
- high quality optical elements for optimized transmission between 1,200-1,700 nm (with optional extension)
- full optical engineering

THIRD HARMONIC GENERATION

Third Harmonic Generation is a specific effect of 3P excitation, that results from the conversion of three incoming photons into one emitted photon with tripled energy and thus, the emission of light of one third the wavelength. THG occurs at structural interfaces that are formed between aqueous fluids and lipid-rich structures, for instance biological membranes, and between water and large protein aggregates, such as collagen bundles or muscle fibers.



THG image of a section of mouse kidney: kidney cells were excited at 1500 nm, and the emitted photons were collected at the green channel (~500 nm) of the detector system.

Femtonics Ltd. HQ
info@femtonics.eu | www.femtonics.eu

Femtonics Inc. USA
usa@femtonics.us

WP-OM-3P-210915

THINKING AHEAD
FEMTONICS
MICROSCOPY