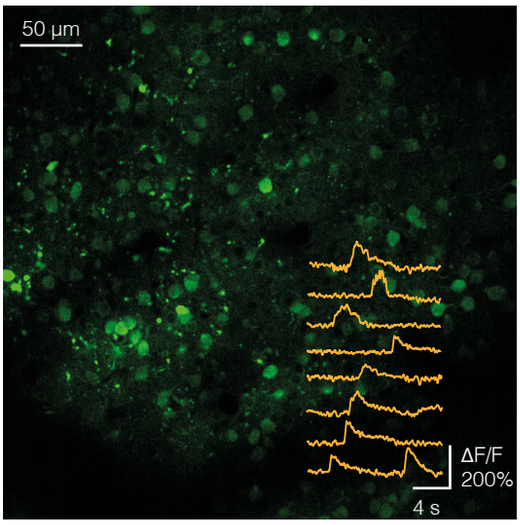
Three-photon excitation

The three-photon (3P) microscopy allows noninvasive functional imaging by making cells visible also in the deeper tissues with high spatial-resolution and better contrast compared to the two-photon excitation.

3P excitation is performed at an excitation wavelength range of 1,200-1,600 nm provided by an amplified laser system. The longer excitation wavelengths are scattered less in the biological tissues, which allows extending the penetration depth, reduces out-of-focus excitation, and increases the signal-to-noise-ratio. The optical design required for 3P excitation establishes higher axial resolution than that of the two-photon microscopy. The spectral window enables 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.



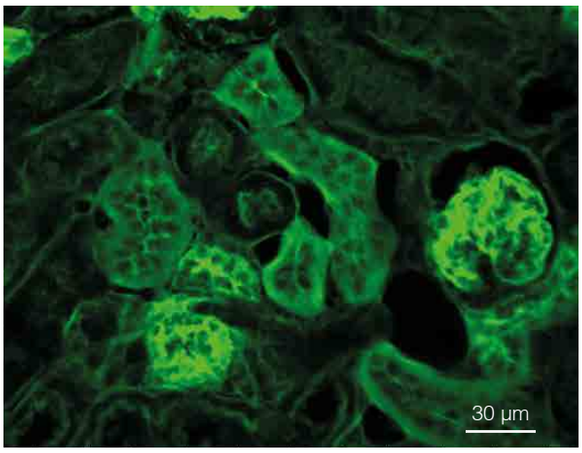
Fluorescent activity measured on several somata from the V1 region of GCaMP labeled mouse

Features

- functional imaging through the whole cortical depth
- XY resolution <500 nm, Z resolution ~ 1 µm
- excites fluorophores in the wavelength range from blue to green
- no noticeable tissue damage

3P upgrade consists of

- Coherent Monaco pump laser and Opera-F optical parametric amplifier system
- high quality optical elements optimized transmission between 1,200-1,600 nm
- full optical engineering



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.

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 the 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.

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