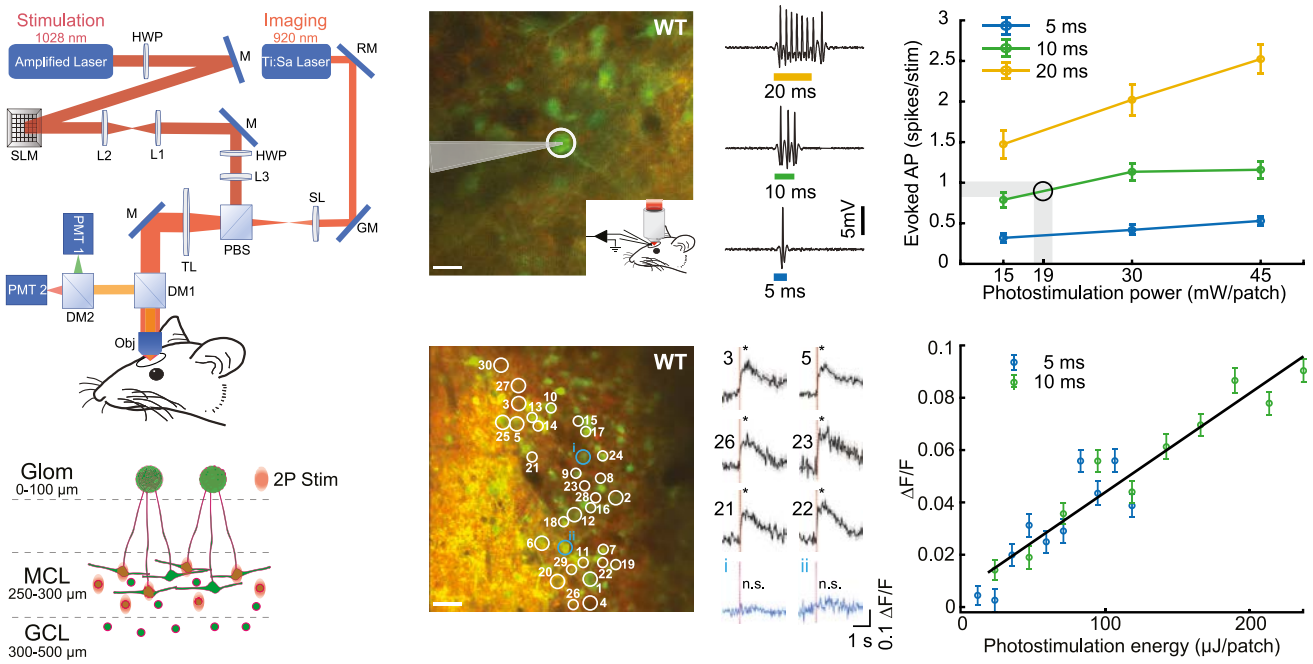
Examples of Microscopy Applications

HOLOGRAPHIC 2P OPTOGENETICS

Traditional full-brain neurostimulation using CW light lacks specificity, whereas point-by-point laser-scanning, despite being highly specific, can be slow and is not simultaneous. Holographic multiphoton neurostimulation, on the other hand, is capable of random-access-style volumetric neuron activation and is therefore used for advanced behavioral neuroscience studies. Holographic stimulation is exceptionally demanding on the laser source, requiring very high average power, combined with complex on-demand pulse train control – features that are well supported by the CARBIDE and PHAROS laser families.

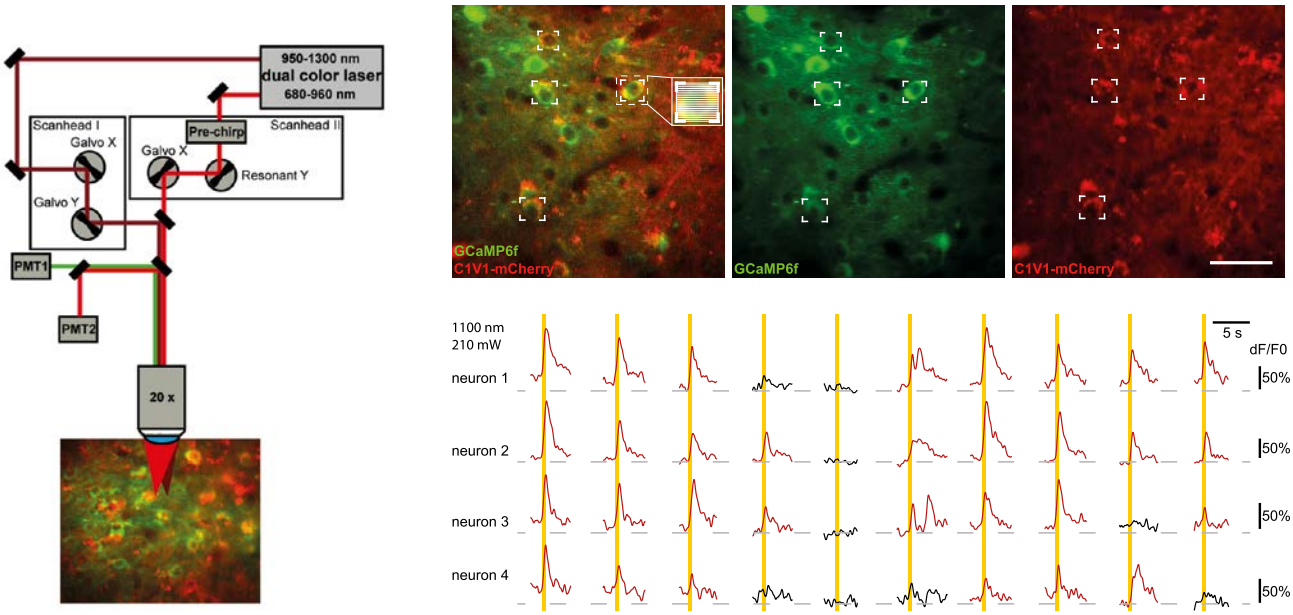


Holographic 2P optogenetic stimulation of mouse olfactory bulb neurons using laser system with PHAROS femtosecond laser.

Courtesy of Shy Shoham and Dmitry Rinberg groups, New York University. Source: J. V. Gill *et al.*, Precise holographic manipulation of olfactory circuits reveals coding features determining perceptual detection, Neuron 108 (2020).

2P OPTOGENETICS

Despite the advances in 3-photon excitation sources providing longer wavelengths and higher pulse energies, certain imaging challenges are still better addressed by tunable high-repetition-rate oscillator-based lasers. This is especially true when imaging speed is the primary factor. For these applications, the CRONUS-2P laser offers the ultimate solution with its optically synchronized three-outputs, two of which are independently tunable. A three-beam source enables a variety of multiphoton excitation pathways, many of which are inaccessible using traditional single- and two-beam solutions. Furthermore, independent tunability of the two beams enables new coherent Raman scattering modalities.

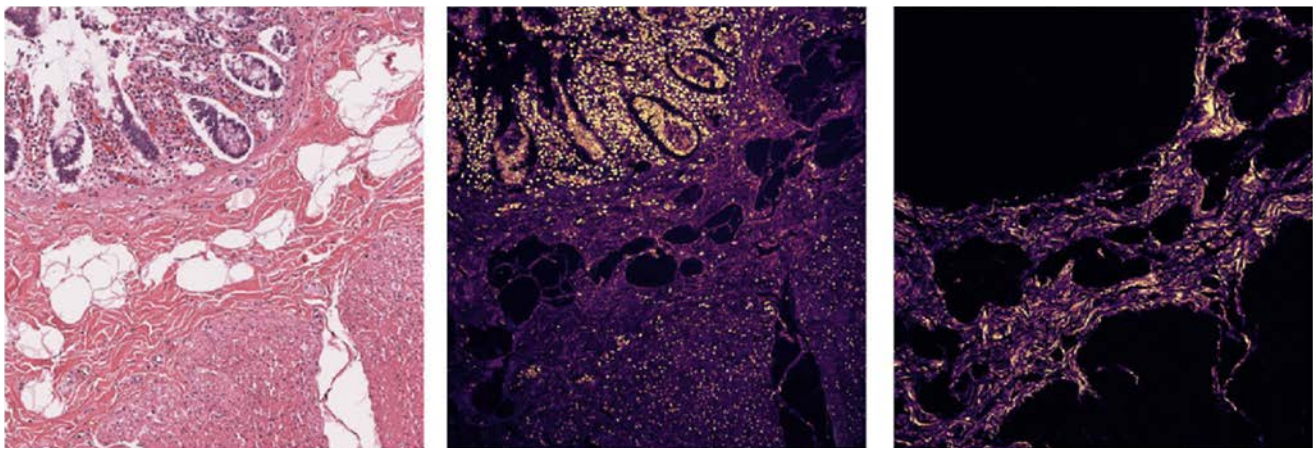


2P optogenetic stimulation of individual neurons using CRONUS-2P.

Courtesy of Albert Stroh group, University Medical Center Mainz and Leibniz Institute for Resilience Research. Source: T. Fu *et al.*, Exploring two-photon optogenetics beyond 1100 nm for specific and effective all-optical physiology, iScience 24 (2021).

RASTER-SCANNING 2P/3P MICROSCOPY

For applications requiring a fixed-wavelength femtosecond laser, such as multiphoton-driven fluorescence (MPEF), excited at 1 µm, and harmonic-generation (SHG, THG) microscopy, the FLINT oscillator is a high-performance solid-state source in a proven, industrial-grade package and a compact footprint. In particular, the FLINT oscillator provides stable 24/7 operation with excellent noise performance, characterized by a RIN that is <140 dBc/Hz above 200 kHz and shot-noise-limited at -160 dBc/Hz above 1 MHz.

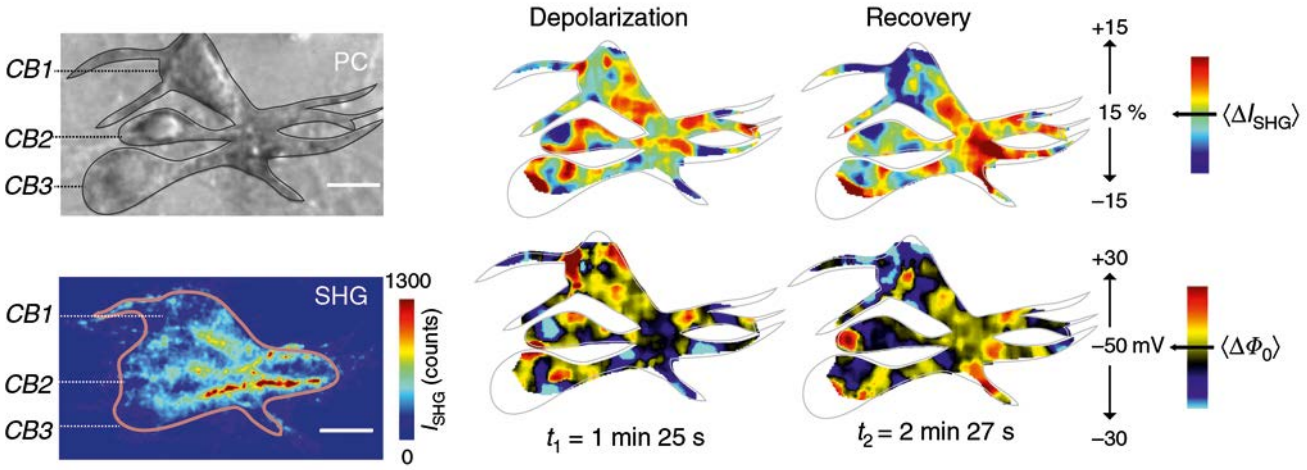


SHG and THG images of H&E-stained colon using FLINT femtosecond oscillator.

Courtesy of Virgis Barzda group, Vilnius University.

WIDEFIELD SHG NEUROIMAGING

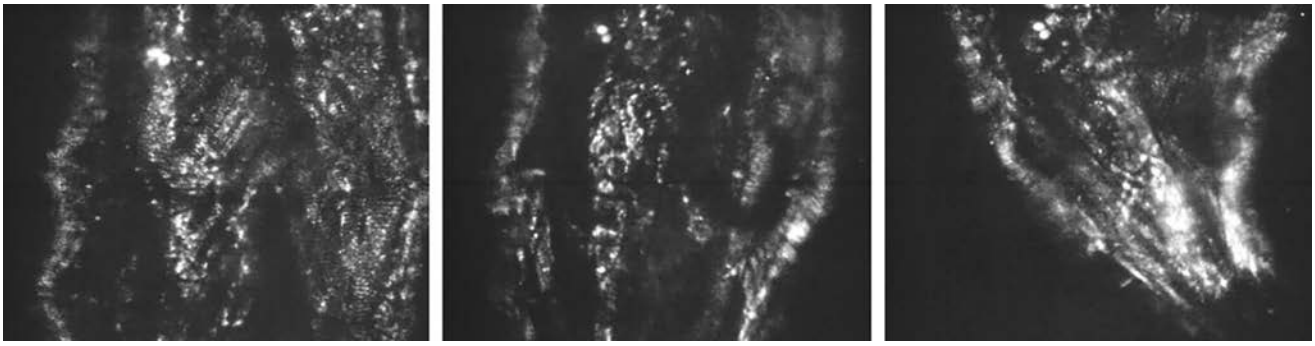
Nonlinear widefield excitation, and in particular second-harmonic generation (SHG) imaging, has a distinct advantage in neuronal voltage potential imaging. This is due to the fact that membrane potential affects the organization of SHG-active molecules and thus SHG imaging enables unprecedented, contactless, real-time voltage imaging at the individual neuron level. Here too, the configuration versatility as well as the low-noise excitation performance of the PHAROS and CARBIDE lasers are very useful.



Widefield SHG neuroimaging of neuronal membrane potentials and ion efflux by means of water using PHAROS femtosecond laser.
Courtesy of Sylvie Roke group, École Polytechnique Fédérale de Lausanne. Source: M. E. P. Didier *et al.*, Membrane water for probing neuronal membrane potentials and ionic fluxes at the single cell level, Nature Communications 9 (2018).

LABEL-FREE *IN VIVO* WIDEFIELD SHG IMAGING

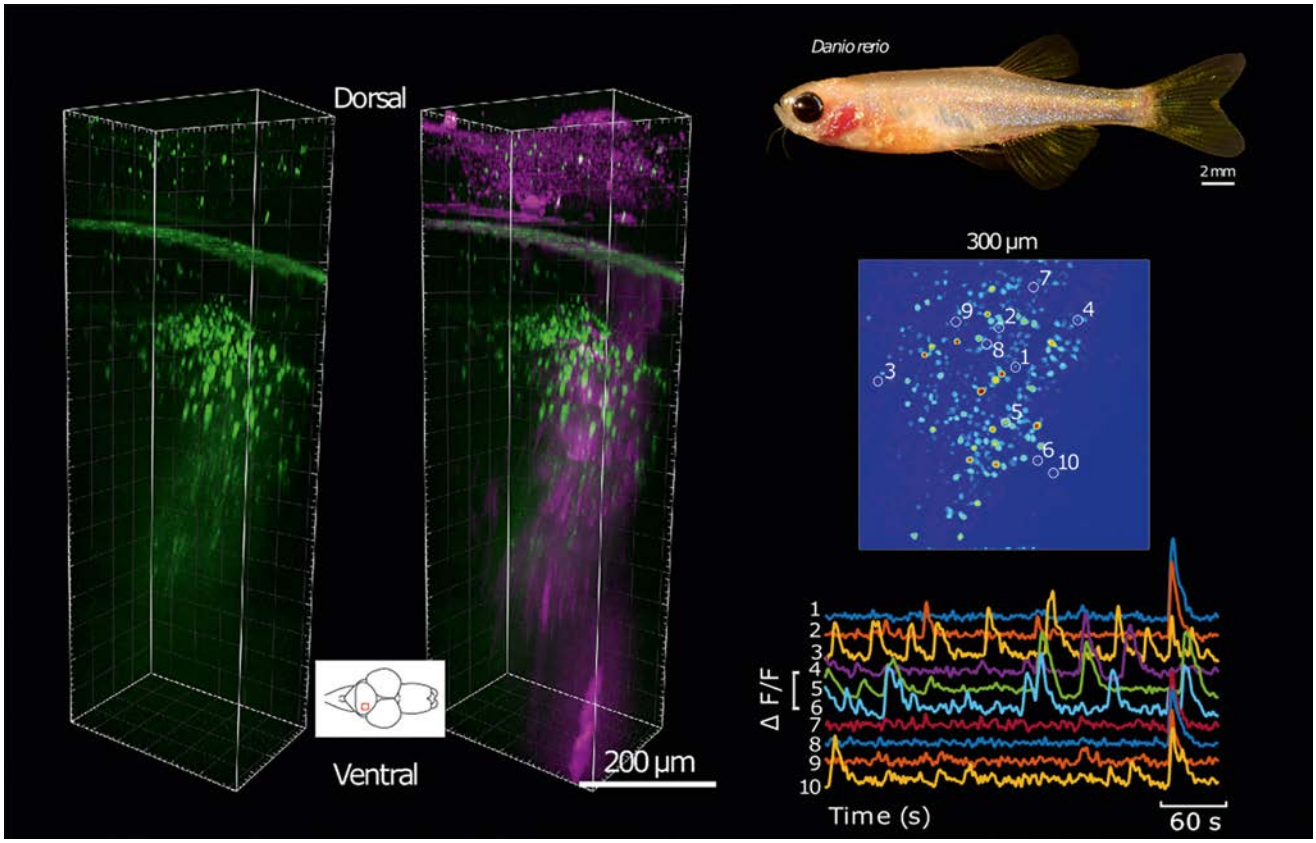
Nonlinear excitation requires very high peak intensities and thus has been traditionally limited to laser-scanning microscopy using tightly focused beams. For some *in vivo* and high-throughput applications, however, laser scanning is too slow. With improved femtosecond laser technology delivering ever-increasing average power, it is now possible to excite nonlinear signals over a large area using widefield nonlinear microscopy. Since optimal excitation conditions are application dependent, the tunable repetition rate and pulse energy of the PHAROS and CARBIDE lasers as well their industrial reliability and low-noise performance are key parameters when building widefield setups.



Label-free *in vivo* widefield SHG imaging of fruit fly larva using PHAROS femtosecond laser.
Courtesy of Virgis Barzda group, University of Toronto.

FUNCTIONAL 3P NEUROIMAGING

Recording of real-time single-neuron activity in the deep brain layers of awake animals is crucial for understanding behavior as well as brain connectivity and function. These applications have been advanced by neuron imaging and stimulation using high-power, high-pulse-energy, medium-repetition-rate lasers tunable in the SWIR range, which spans the biological transparency windows at 1.3 µm and 1.7 µm. For two- and three-photon-excited fluorescence (2PEF, 3PEF) and harmonic-generation (SHG, THG) imaging in deep tissues, dispersion-controlled femtosecond pulses from ORPHEUS line OPAs and microscopy-dedicated CRONUS-2P and CRONUS-3P lasers are truly a state-of-the-art choice.



Functional three-photon neuroimaging of zebrafish using OEM OPA in ORPHEUS-F configuration.

Courtesy of Chris Xu and Joe Fetcho groups, Cornell University. Source: D. M. Chow *et al.*, Deep three-photon imaging of the brain in intact adult zebrafish, Nature Methods 17 (2020).