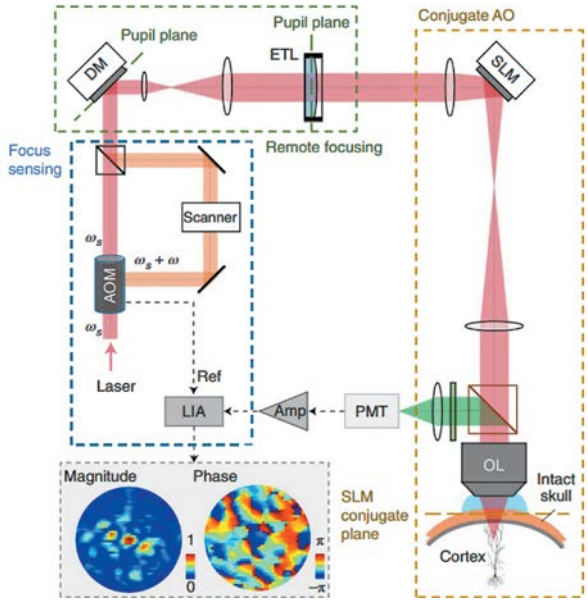
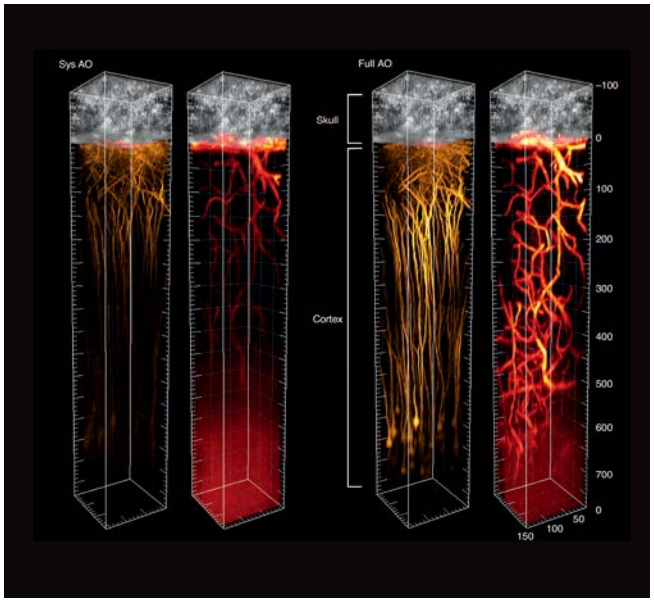
Nonlinear Microscopy

Functional 3P neuroimaging

Recording real-time single-neuron activity in the deep brain layers of awake animals is essential for understanding behavior, brain connectivity, and function. These applications have been advanced by neuron imaging and stimulation techniques using high-power, high-pulse-energy lasers with medium-repetition rates, tunable in the SWIR range, which aligns with the biological transparency windows at 1300 nm and 1700 nm. For 2P and 3P excited fluorescence, and harmonic-generation (SHG, THG) imaging in deep tissues, dispersion-controlled femtosecond pulses from **I-OPA** and **ORPHEUS** OPAs and microscopy-dedicated **CRONUS** lasers represent state-of-the-art choices.





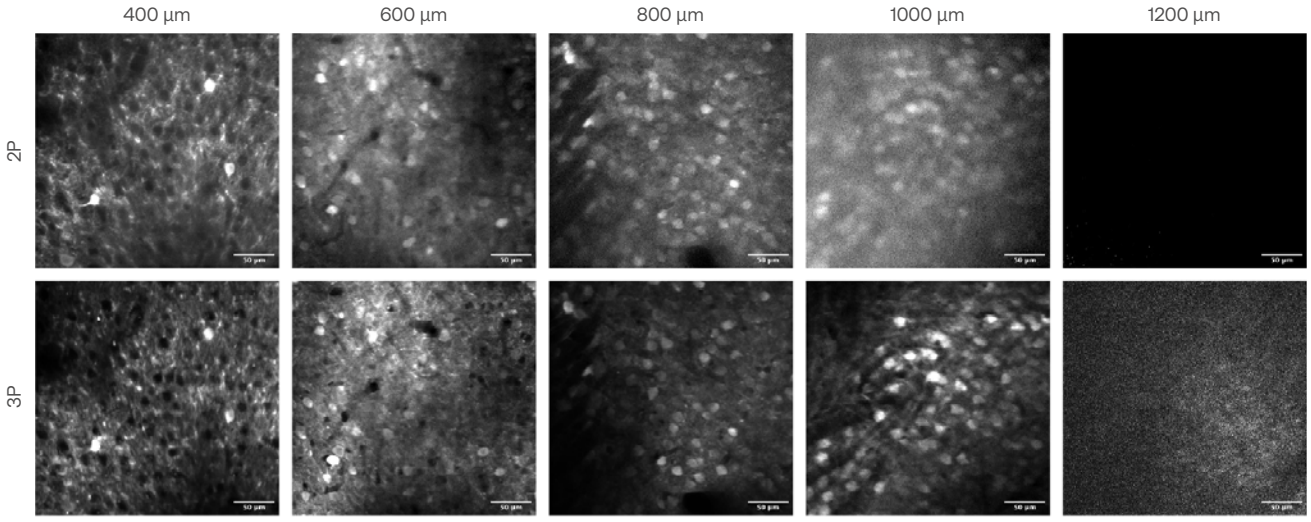
3P microscopy with adaptive optics for focus sensing and shaping to compensate for both aberrations and scattering. **ORPHEUS-F** excitation at 1300 nm enabled imaging up to 1.1 mm below the pia within the intact brain.

Courtesy of Jianan Y. Qu group, the Hong Kong University of Science and Technology. Source: Qin et al., Deep tissue multi-photon imaging using adaptive optics with direct focus sensing and shaping, Nature Biotechnology 40 (2022).

2P and 3P calcium imaging at depth in mouse brain

Three-photon microscopy (3PM) has gained popularity as a tool able to extend the capabilities of two-photon microscopy (2PM) by imaging deeper layers in the brain and other tissues such as tumors and bone.

Imaging depth in 2PM is limited by the scattering and absorption of excitation light within the tissue. 3PM overcomes this limit because the higher nonlinearity of the 3P excitation reduces the background.

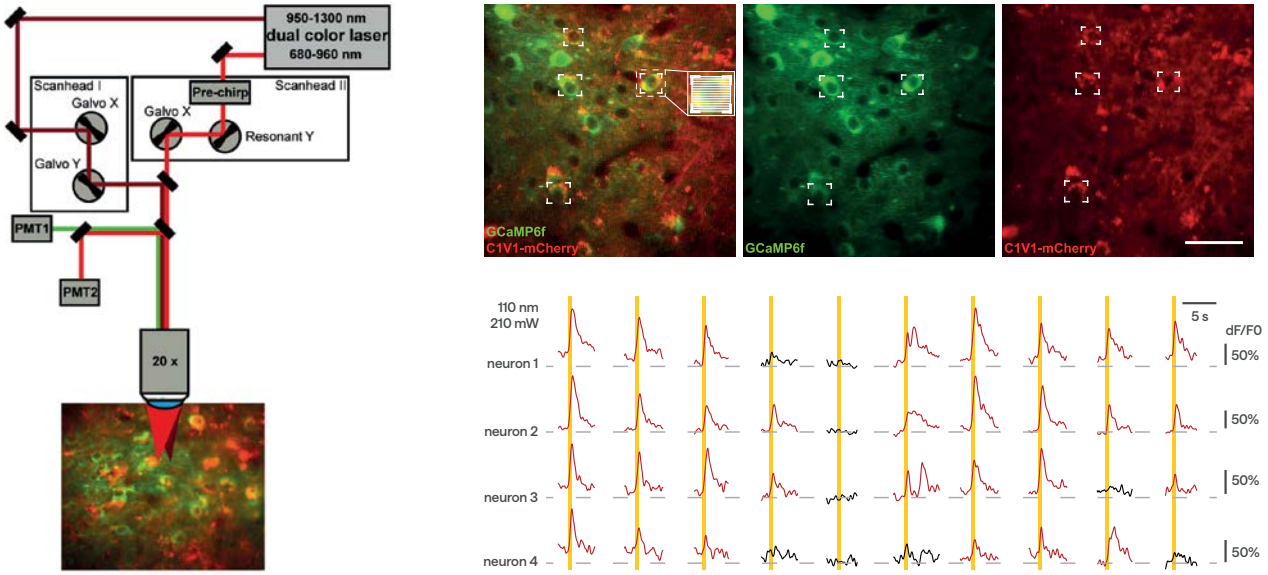


Comparison of in vivo 2P and 3P calcium imaging of mouse visual cortex GCaMP neurons on a Thorlabs Bergamo II microscope using a typical 2P laser and Light Conversion's **CRONUS-3P** (3P) laser at 920 nm and 1300 nm, respectively.

Courtesy of CSHL ISFNS 2024 school organizers, Willis Broden Jr. and Sergey Matveev (Thorlabs).

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, the independent tunability of the two beams enables new coherent Raman scattering modalities.

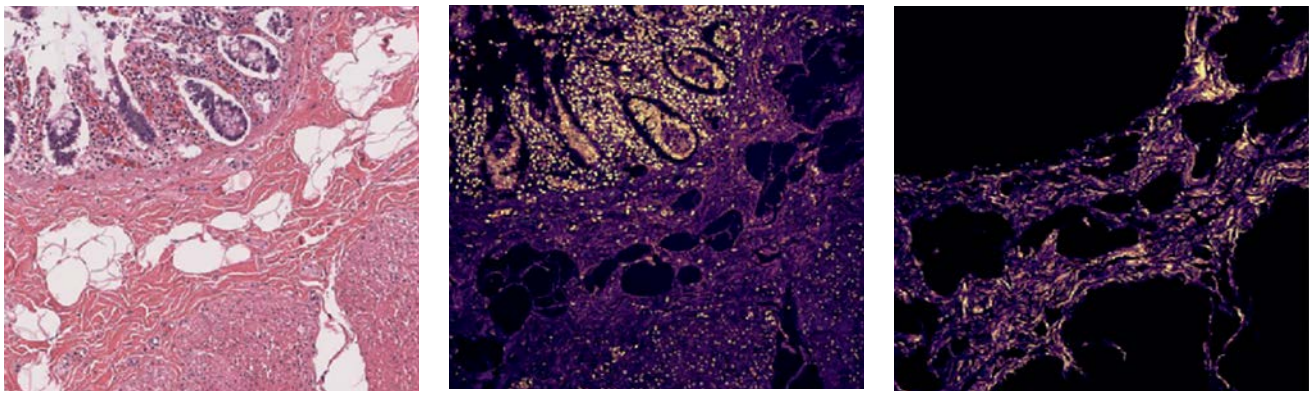


2P optogenetic stimulation of individual neurons using **CRONUS-2P**.

Courtesy of Albrecht 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, 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. The **FLINT** oscillator provides stable 24/7 operation with excellent noise performance, characterized by a RIN of < 140 dBc/Hz above 200 kHz and shot-noise-limited performance at -160 dBc/Hz above 1 MHz.



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

Courtesy of Virginijus Barzda group, Vilnius University.