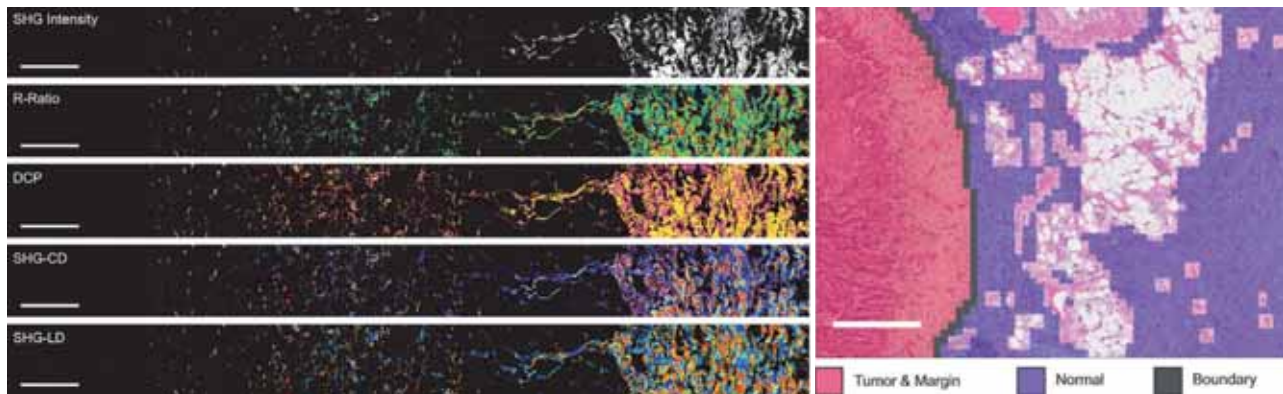


# Nonlinear Histopathology

## Widefield polarimetric SHG microscopy

Cancer diagnosis and surgical treatment rely on imaging techniques that demand specificity and high throughput. Polarization-resolved second-harmonic generation (P-SHG) microscopy shows potential for visualizing structural changes in collagen networks and the extracellular matrix associated with tumor development. Moreover, P-SHG imaging is label-free and compatible with live tissue imaging at depth. However, traditional raster scanning methods are too slow for clinical applications, and interpreting the structural sensitivity of P-SHG can be challenging.

Nonlinear widefield microscopy addresses these limitations by utilizing amplified femtosecond lasers to increase imaging throughput and field of view. Additionally, machine learning (ML) techniques enable data-driven analysis, facilitating tasks such as automating tumor margin delineation and scoring. By leveraging **CARBIDE** and **PHAROS** lasers in conjunction with ML-augmented widefield microscopy, we can potentially extend the benefits of nonlinear microscopy to the scale required for biomedical and clinical applications.



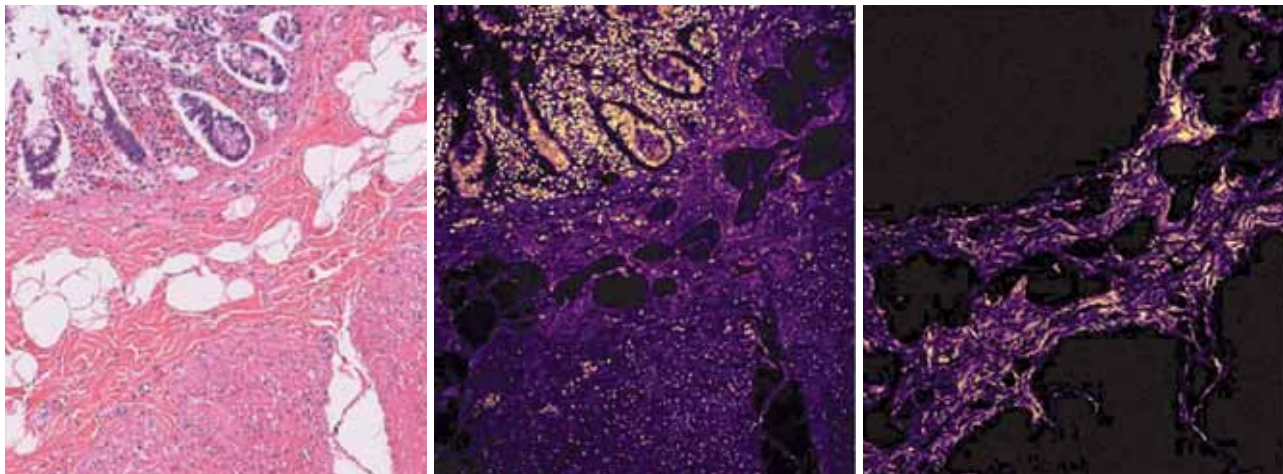
Large-area widefield P-SHG microscopy of human lung tissue tumor margins conducted using the **PHAROS** laser. Image parameters, including SHG intensity, R-ratio, and degree of circular polarization, as well as SHG circular and linear dichroism, are employed in unsupervised ML algorithms to determine the tumor boundary.

Courtesy of Virginijus Barzda group, University of Toronto, and Brian C. Wilson group, Princess Margaret Cancer Centre. Source: Mirsanaye et al., Unsupervised determination of lung tumor margin with widefield polarimetric second-harmonic generation microscopy, *Scientific Reports* 12 (2022).

## Raster-scanning SHG/THG microscopy

For applications requiring a fixed-wavelength femtosecond laser, such as multiphoton-driven fluorescence, excited at 1  $\mu\text{m}$ , 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, providing a stable 24/7 operation with excellent noise performance.



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

Courtesy of Virginijus Barzda group, Vilnius University.

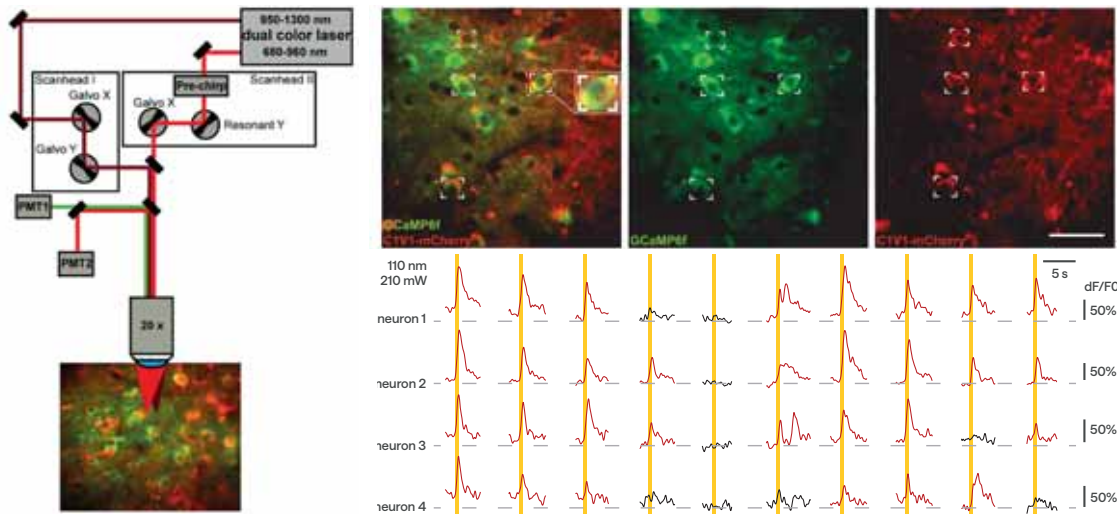


## All-optical physiology

Despite the ability of 3-photon imaging to provide high-contrast within deep tissue, many experimental questions are still better addressed with 2-photon imaging – particularly when video-rate acquisition is required. For these applications, the **CRONUS-2P** laser offers the ultimate solution, featuring three optically synchronized outputs, two of which are independently tunable. This three-beam source enables

a wide range of multiphoton excitation pathways, many of which are inaccessible with traditional single- or dual-beam solutions.

For experiments that require simultaneous activation of large numbers of cells, the **CARBIDE** laser remains a proven, high-performance choice for demanding two-photon optogenetics.



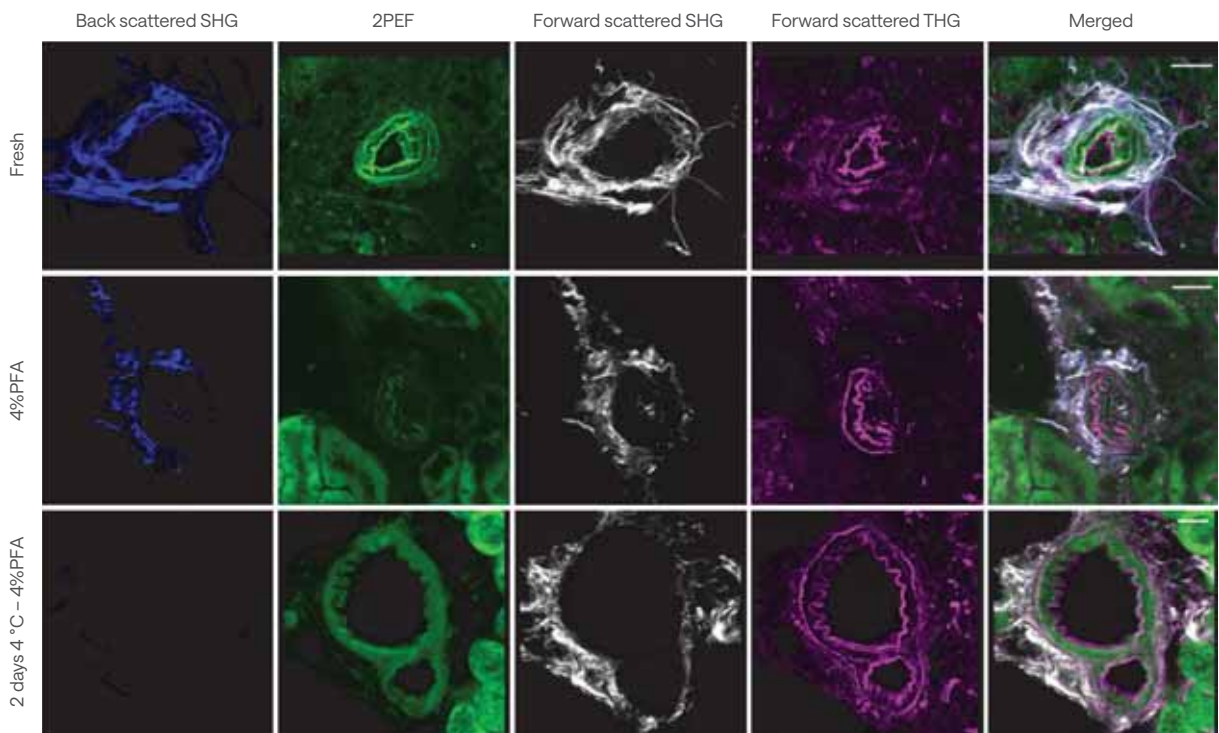
2P optogenetic stimulation of individual neurons using **CRONUS-2P**.

Courtesy of Albrecht Stroth 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).

## SHG, THG, and 2P imaging

Fixation methods, such as formalin, are commonly used to preserve tissue and keep its structure as close as possible to the native condition. However, fixatives chemically interact with tissue molecules and may modify their structure. Taking advantage of the SHG and THG emission capabilities of such components, nonlinear

2P microscopy and the **CRONUS-2P** femtosecond laser were used to evaluate the effect that preservation methods, such as chemical fixatives, have on the nonlinear capabilities of protein components within mouse tissues.



SHG signals from collagen, 2PEF and THG signals from elastin in vibratome sections of mouse kidney after different treatments, acquired using the **CRONUS-2P** femtosecond laser source.

Courtesy of Frauke Alves and Fernanda Ramos-Gomes, Max-Planck Institute for Multidisciplinary Sciences, Germany.