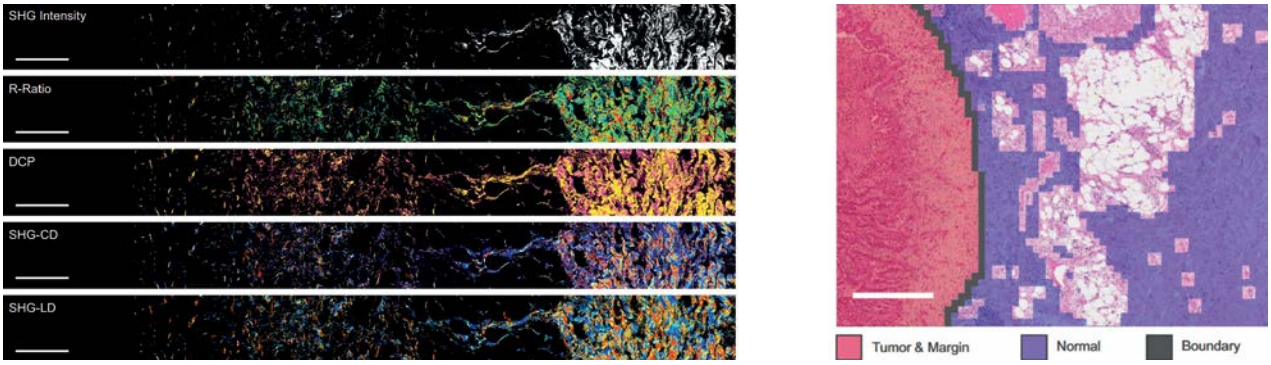
Widefield polarimetric SHG microscopy

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 **PHAROS** and **CARBIDE** 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.

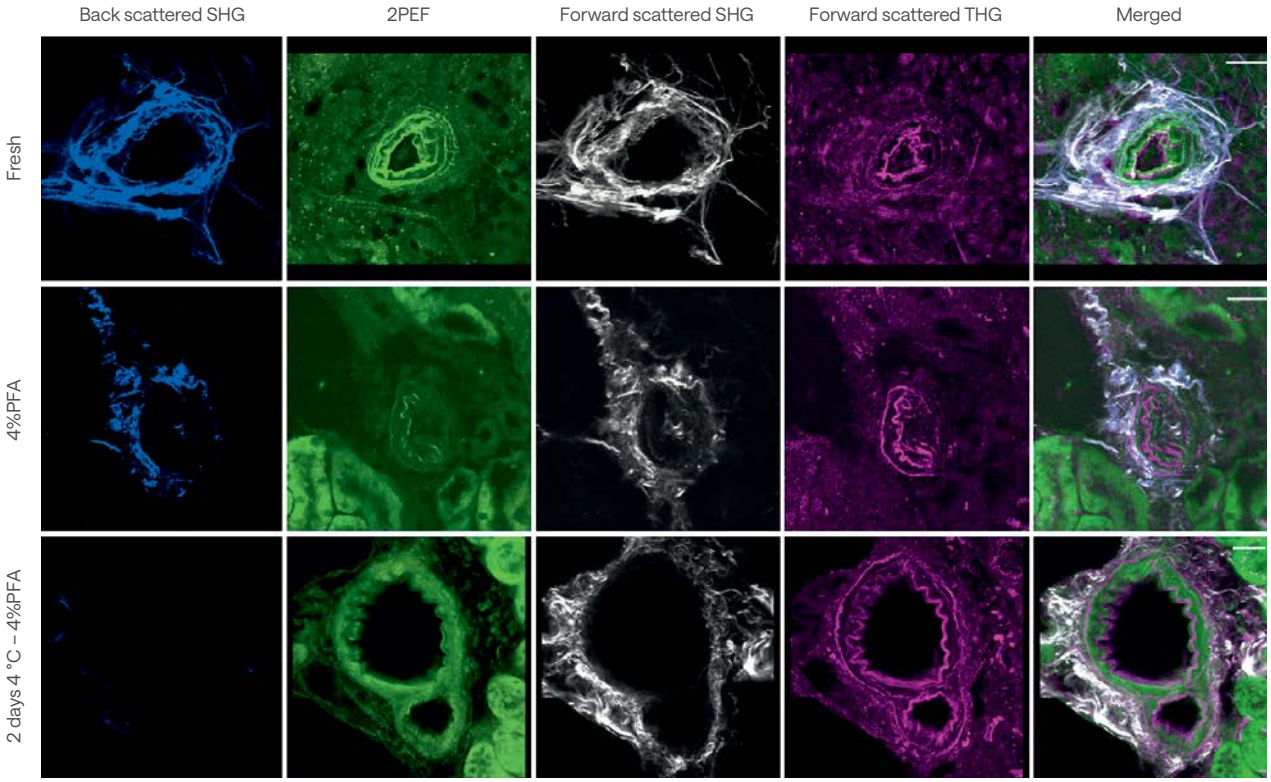


Large-area widefield P-SHG microscopy of human lung tissue tumor margins conducted using the **PHAROS** laser. Image parameters, including SHG intensity, R-ratio, and degree of circular polarization, as well as SHG circular and linear dichroism, are employed in unsupervised ML algorithms to determine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

SHG, THG, and 2P imaging

Fixation methods, such as formalin, are commonly used for tissue preservation to maintain their structure as close as possible to the native condition. However, these fixatives chemically interact with tissue molecules, potentially altering their structure. To assess the impact of preservation methods, such as chemical fixatives, on the nonlinear capabilities of protein components within mouse tissues, nonlinear two-photon (2P) microscopy and the **CRONUS-2P** femtosecond laser were utilized. These techniques take advantage of the SHG and THG emission properties of tissue components.



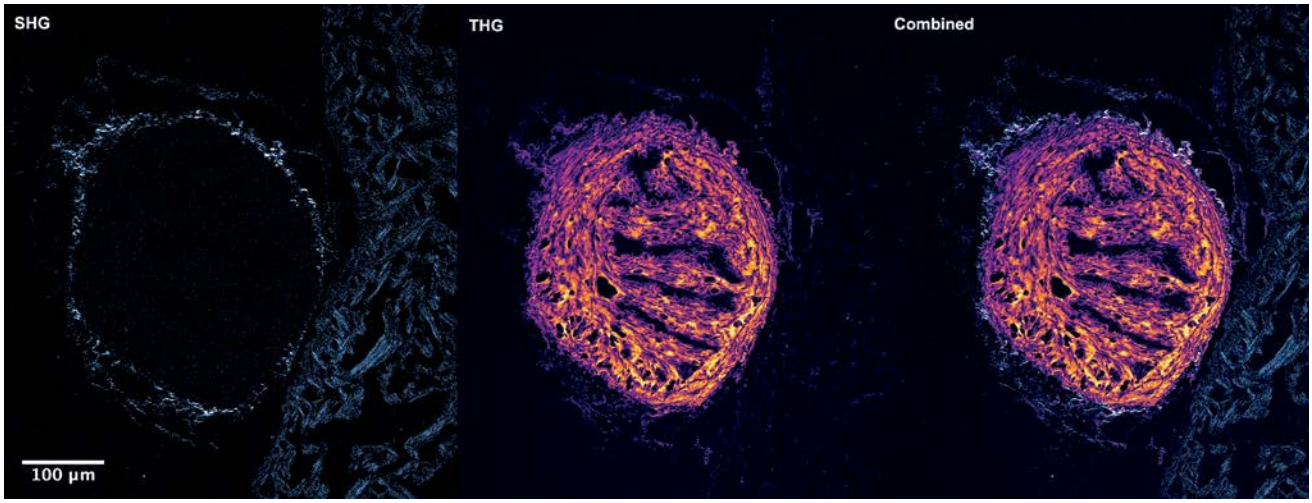
SHG signals from collagen, 2P excitation microscopy and THG signals from elastin in vibratome sections of mouse kidney after different treatments, registered using the **CRONUS-2P** femtosecond laser source.

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

Combined SHG and THG imaging

Adult zebrafish heart ventricle section used in a scar formation study imaged with the **FLINT** femtosecond oscillator. The brightfield image is stained with Masson's trichrome (MT), where connective tissue appears blue and muscle appears red/brown.

SHG and THG images reveal collagen and muscle structure at the periphery of the bulbus arteriosus, while MT-stained elastin is visualized in the center in THG.



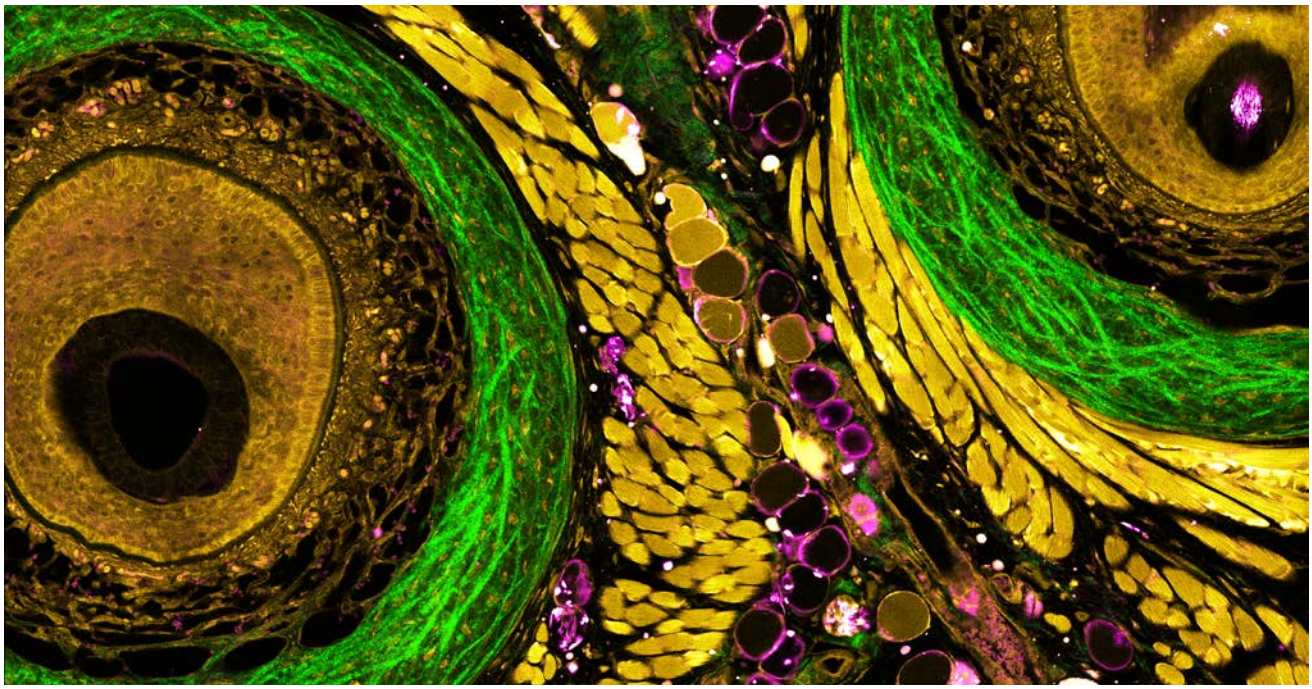
Adult zebrafish heart ventricle section imaged using the **FLINT** femtosecond oscillator.

Samples courtesy of Justas Lazutka at the Vilnius University Life Sciences Center. Nonlinear imaging courtesy of the Virginijus Barzda group at the Vilnius University Faculty of Physics.

Label-free in vivo imaging

Understanding biological complexity requires minimally disruptive imaging tools capable of providing multiplexed molecular contrasts. To address this need, S. You's laboratory at the Massachusetts Institute of Technology is developing a non-invasive, label-free microscopy approach using CRONUS-3P to visualize biosystems.

As part of a study on neuropathic pain, the image reveals the rich microenvironment of an unprocessed, intact mouse whisker pad: collagen capsule (green), comprising the follicle with muscles (yellow) supporting it, adipocytes (purple), stromal cells, and immune cells.



Mouse whisker pad using label-free microscopy.

Courtesy of Sixian You group, Massachusetts Institute of Technology.