FXS-DUAL – ULTRAFAST FEMTOSECOND LASER FOR CARS MICRSOCOPY

Thomas Kellerer[1], Patrick Byers[1], Stefanie Kiderlen[2] and Lukas Krainer[2]

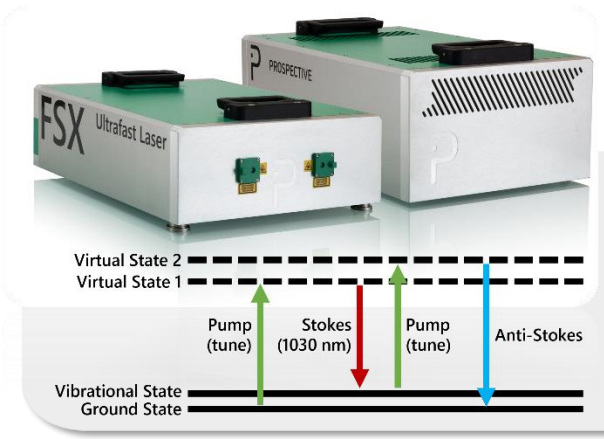
1. Multiphoton Imaging Lab – University of Applied Sciences Munich - Germany
2. Prospective Instruments LK OG, Dornbirn – Austria

Coherent anti-Stokes Raman Scattering (CARS) microscopy is an imaging technique frequently used in biomedical research and materials science. CARS microscopy is a third-order nonlinear optical microscopy technique that enables label-free imaging of samples with high spatial resolution and chemical specificity. In the field of biology, CARS microscopy is mainly used for label-free imaging of lipids in cells, tissues and whole animal models such as *Caenorhabditis elegans* (C. elegans).

The identification and imaging of label-free biomarkers has spread rapidly in the field of life science research in recent years. Detailed observation at the molecular level is crucial for basic research, understanding mechanisms behind diseases such as metabolic disorders [1] or Alzheimer's disease [2], or diagnosing biopsied and resected tissue. Here, time-saving, depth-resolved, and sample-saving noninvasive data acquisition is of great advantage. Some nonlinear optical modalities are suitable for this purpose, such as two-photon, three-photon, second-harmonic generation (SHG), third-harmonic generation (THG), coherent anti-Stokes Raman spectroscopy (CARS), stimulated Raman spectroscopy (SRS), and fluorescence lifetime imaging (FLIM) employed (REF.). They all have their own advantages and disadvantages; however, combining the modalities maximizes the information content and provides complementary data [3,4].

CARS is a third-order nonlinear process that occurs when a sample is illuminated by two laser beams of different wavelengths (the so-called *"Pump"* and *"Stokes"* beams) that are synchronized in time and space. As a result, the sample is excited by a wave mixing process of both laser beams. The contrast of CARS microscopy is obtained when the frequency difference **Δω=ωp- ωs** between the pump beam and the Stokes beam is equal to the frequency of molecular vibration of a particular chemical bond.

The pump beam creates virtual stages in the sample, while the Stokes beam, which hits the sample simultaneously, stimulates the population of virtual stages. The simultaneous illumination of the pump and Stokes beams creates a dense population of



"FSX-Dual – Ultrafast Dual-Color Femtosecond Laser for CARS Microscopy"

The **FSX-Dual** with two wavelengths, one at **1030 nm** and the other tunable between **760 and 940 nm** is a versatile, turn-key and compact femtosecond laser perfectly matched for multiphoton imaging and other biophotonic applications like **2P/SHG/THG, CARS/SRS, FLIM** and many more.

a vibrational sub-state of the ground state, which is very specific to the chemical properties. Which molecules or chemical properties are addressed here is determined by the tunable pump laser in FSX-Dual. By continuously illuminating the sample with Pump- and Stokes-beam at energy difference Δω, the vibrational states of the molecules are driven in a coherent superposition of states. Energy released from the oscillating bonds generates a blue-shifted Anti-Stokes emission by combining the energy of the vibrational state and an additional Pump-photon. This now forms the imaging contrast.

OPTICAL SETUP: FSX IN AN OPEN TABLE MICROSCOPE

Prospective's FSX-Dual laser with a fixed wavelength at 1030 nm and a tunable wavelength between 760 nm and 940 nm allows CARS microscopy preferably at wavenumbers around 2850 cm^{-1}, where especially lipids can be imaged label-free. To synchronize the two output laser beams in time, one of the two beams is delayed via a time delay that both beams hit the sample at time zero and generate a CARS signal.

"The FSX-Dual – Open Table Setup for CARS Microscopy"

Figure 1: Prospective's FSX dual coupled in an optical path of an open table microscope. The fixe Stokes laser beam at 1030 nm was running over a time-delay (Thorlabs-Stage with movable mirrors) and the tunable Pump beam was set to 794 nm for label-free imaging of fat and lipids with wavenumbers at 2850 cm^{-1}.

Technical supervision and implementation were done by Prof. Thomas Hellerer and Thomas Kellerer, Multiphoton Imaging Lab Munich.

LABEL-FREE CARS MICROSCOPY OF FAT-TISSUE

CARS microscopy allows label-free and non-destructive analysis of adipose tissue since it can image lipid molecules at a wave number of 2850 cm^{-1}. Adipose tissue is composed primarily of adipocytes, specialized cells that store energy in the form of triglycerides. CARS microscopy can provide valuable insights into the structure and composition of adipose tissue at the molecular level, allowing researchers to investigate the structure, composition, and metabolic processes in healthy and diseased tissue.

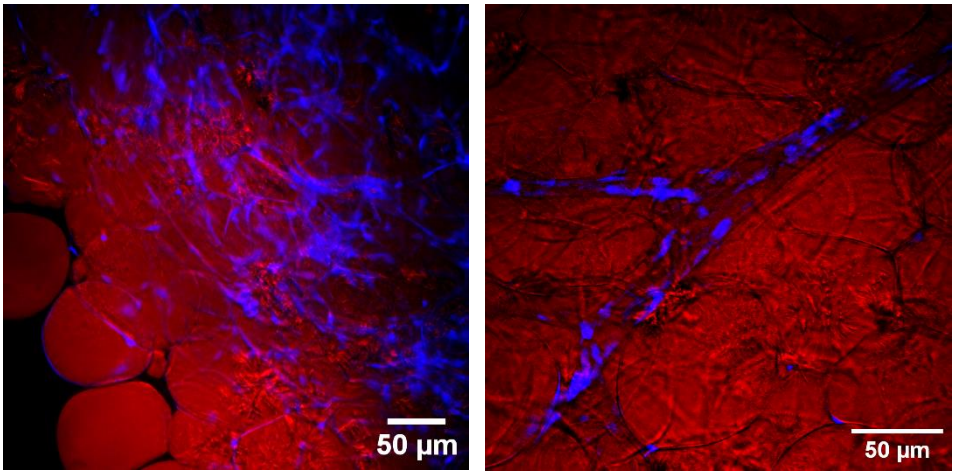


Figure 2: Cryosection of mouse adipose tissue showing the label-free CARS signal in red and a nuclear DAPI stain in blue. Image courtesy Thomas Kellerer (HM Munich).

LABEL-FREE CARS MICROSCOPY OF LIVING C.ELEGANS

CARS microscopy is also used to study the C. elegans, a model organism widely used in biological research. C. elegans is a transparent organism that offers unique advantages for microscopic imaging. CARS microscopy can provide valuable insights into its molecular composition and physiological processes. Label-free imaging of lipid-rich structures in C.elegans, such as lipid droplets and membranes, enables the study of spatial information about lipid distribution and dynamics, lipid-rich neuronal structures, enabling the study of neural development, connectivity, and neurodegenerative processes, lipid metabolism or lipid homeostasis.

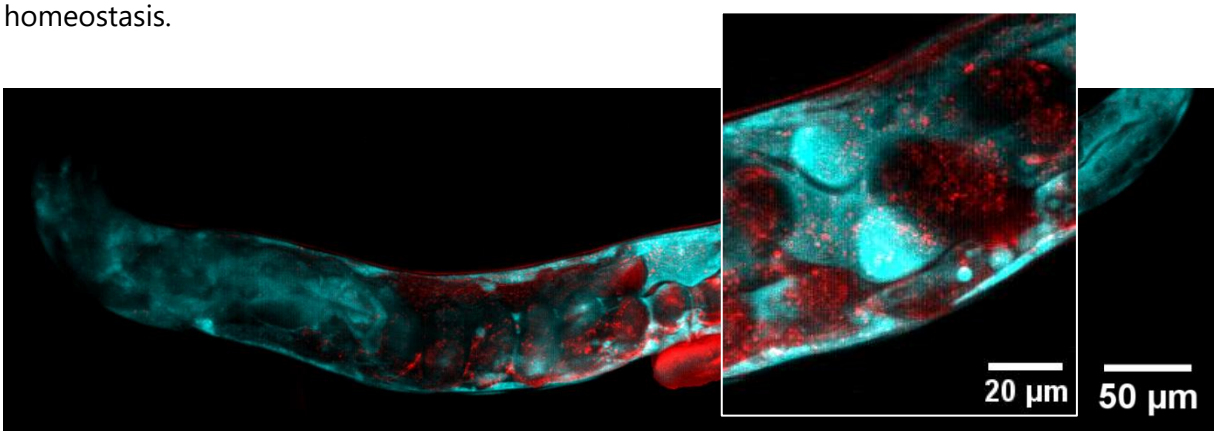


Figure 3: In-vivo of a C. elegans showing the label-free CARS signal (red) and GFP-tagged ribosomes in all somatic tissues (cyan). Image courtesy Prof. Carmen Nussbaum, LMU Munich and Thomas Kellerer, HM Munich

Acknowledgements

We kindly acknowledge Prof. Thomas Hellerer, Multiphoton Imaging Lab (University of Applied Sciences Munich) for his technical support and for testing our laser with his microscopy setup, Prof. Carmen Nussbaum and Dr. Jessica Tittelmeier (Ludwig-Maximilians Universität Munich) for providing C. elegans samples.

References

[1] Hellerer et al.: Monitoring of lipid storage in Caenorhabditis elegans using coherent anti-Stokes Raman scattering (CARS) microscopy (2007), PNAS

[2] Luo et al.: Long-Term Repeatable In Vivo Monitoring of Amyloid-β Plaques and Vessels in Alzheimer's Disease Mouse Model with Combined TPEF/CARS Microscopy (2022), MDPI

[3] Perry et al. Two-Photon and Second Harmonic Microscopy in Clinical and Translational Cancer Research (2012), Annals of Biomedical Engineering

[4] Strachan et al.: Pharmaceutical applications of non-linear imaging (2010) Pharmaceutical applications

CONTACT:

Keep up-to-date: Visit our website or follow us on social media.

EU

Prospective Instruments LK OG
6850 Dornbirn, Austria
Email contact@p-inst.com
www.p-inst.com

SWITZERLAND

Prospective Instruments GmbH
8105 Regensdorf, Switzerland
Email contact@p-inst.com
www.p-inst.com

USA

two-level systems
Allentown, PA 18101
Email info@two-levelsystems.com
www.two-levelsystems.com