Leica TCS CARS

Live Molecular Profiling

Living up to Life
• Imaging without dyes
• Video rate and high resolution in one system
• Easy experiment setup with a fully integrated system
• Excellent imaging with VIS, UV, IR, SHG and CARS
• Leica TCS SP5 II upgrade to CARS anytime
Conventional microscopy techniques depend on the availability of fluorescent dyes and biological structures that can be labeled or show inherent fluorescence, such as autofluorescence. For new applications, new labels are often needed to visualize interesting details.

The limits of staining methods are obvious: dyes bleach with time and alter the specimen. Exposure to atmospheric conditions, or influence by moisture, air or solvents render stained specimens unsuitable for long-term experiments. Furthermore, dyes often cause phototoxicity, harm the cells, and consequently influence the result of the experiment. They can camouflage interesting information and lead to incorrect conclusions.

Besides, specimen preparation can be time consuming. Some samples can’t be stained and analyzed at all because the appropriate dyes are not yet available.

Leica TCS CARS

Live Molecular Profiling

With the integration of CARS technology into the broadband confocal system, Leica TCS SP5 II offers an efficient way to overcome the limitations of dye-based imaging methods. CARS (Coherent anti-Stokes Raman Scattering) microscopy is a label-free technology that images the characteristic intrinsic vibrational contrast of the molecules in a sample. The contrast is no longer caused by staining, but by the molecules themselves. The specimen remains almost unaffected.

The CARS signal is generated by IR excitation only at the focus of the laser beam and leads to 3D sectioning capability. The result is highly sensitive imaging at sub-cellular resolution without any staining.

CARS technology perfectly complements the needs of scientists analyzing living cells or small animals, because the method is non-toxic, non-destructive, and minimally invasive.

With Leica TCS CARS new fields of application become accessible.
Imaging whole organs

Unstained skin of a mouse ear imaged with Leica TCS CARS. Sebaceous glands are multicellular compartments, which contain triglycerides and wax ester. While non-lipid components appear as dark areas in the image, the fatty components are much brighter. Due to their CH₂ bonds they can be perfectly imaged with CARS technology and lead to sharp, crisp results.

Courtesy of Prof. Sunney Xie, Department of Chemistry and Chemical Biology, Harvard University, Cambridge, USA

Food analysis

Leica TCS CARS visualizes the fatty components in food. The maximum projection shows lipid components (red), which are located not only at the surface but also inside a potato chip. Green parts in the image provide structural information generated by multiphoton fluorescence signals only.
Imaging complex structures

The maximum projection of a *Plodia interpunctella* is imaged label-free with CARS and multiphoton excitation. The overlay image combines information about lipid localization (red areas) and structural properties (green areas) of the specimen.

Analysis of living cells

Yeast cells are highly environmental-sensitive and do not survive at suboptimal conditions. With Leica TCS CARS they can be imaged without complex treatment of the cells. Left: one week old yeast cells. Medium was not changed during this time. Right: 4 hours old yeast cells in a fresh medium.
“Label-free imaging at video rate is what truly advances today’s research.”

Prof. Eric Olaf Potma, Natural Sciences II
University of California, Irvine, USA

CARS Applications

Leica TCS CARS covers a wide range of application fields:

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<td>Lipid pathways</td>
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<td>Interactions between different compounds and interfaces</td>
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<td>Compound tracking/tracing</td>
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<td>Development of healthcare products</td>
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Listen to vibrating molecules

The CARS signal is generated from vibrational motion of the molecules in the specimen. No additional markers or staining are required to get a CARS image.

Different types of molecules show characteristic vibrational energy states. Electromagnetic energy at appropriate (infrared) wavelengths excite the molecules into these states. To generate sufficient signal above noise, CARS populates a characteristic vibrational state by “pumping” first from ground state into a virtual state. This is achieved by illumination with a “pump” beam at medium wavelength ($\omega_P$). The energy of the photons must be less than the difference from ground state to the first excited state. Upon simultaneous illumination by a second beam (Stokes beam) at longer wavelength ($\omega_S$), the molecule is forced from the virtual state into the desired vibrational state.

As the pump beam is tunable, the difference $\omega_{\Delta} = \omega_P - \omega_S$, can be specifically adjusted to the desired vibrational energy of the molecule of interest. In essence, the desired molecule population is converted into a vibrational state.

To visualize the molecules, the pump beam raises the electronic system into a second virtual state of the energy $\omega_P + \omega_{\Delta}$. From here, the molecule is allowed to relax back to the ground state, while the energy $\omega_P + \omega_{\Delta} = \omega_{AS}$ is detected (anti-Stokes beam). These photons are used for imaging.

Modes of CH₂ vibrations

Symmetrical stretching  Anti-symmetrical stretching  Scissoring
Rocking  Wagging  Twisting

Six typical vibration modes of a CH₂ molecule

Leica Microsystems has solved the critical opto-mechanical challenge of combining a single-point confocal beam with CARS illumination.
To generate a CARS image of a specimen, a perfectly aligned setup of the laser beams is required. Leica TCS CARS and the picoEmerald from APE are a perfect combination in this respect.

A safety box laser solution provides both the pump and the Stokes beam. Both beams are guided through the optimized beam routing, pass through the scanner and the objective, and finally excite the sample. With the integrated AOTF (Acousto-Optical Tunable Filter), the intensity of the beams can be individually attenuated to suit the experiment.

CARS is generated by a non-linear multi-photon process and consequently allows optical sectioning without need of a pinhole. Therefore, the use of non-descanned detectors significantly increases the collection efficiency.

Two detection modes show excellent results: Non-Descanned Detectors (NDDs) collect the signal either in forward direction (transmitted light path) detecting the F-CARS signal or in the epi-direction (reflected light path) detecting Epi-CARS signal.

The filter cubes for both modes are available and optimized for various needs. Thin specimens are perfectly visualized in F-CARS mode while whole organs or small living animals are optimally imaged in the Epi-CARS mode.

Leica TCS CARS provides a picosecond infrared laser, which reduces photodamage of specimens and allows imaging between wave numbers of 1200 cm⁻¹ to 3500 cm⁻¹ with excellent spectral resolution of 2 to 3 cm⁻¹. Images acquired with the picosecond laser are crisper and have better contrast as they show less non-resonant background than CARS images taken with a femtosecond laser.

Setup of the Leica TCS CARS Confocal System

CARS workflow

- Place the unstained specimen on the stage
- Select the wave number according to the specimen (pump-based or Raman-based mode)
- Activate the NDDs
- Select desired scan mode
- Adjust settings such as scan speed and scan format
- Capture an image or start a series acquisition
The Stokes beam ($\omega_S$) and the tunable pump beam ($\omega_p$) are generated by a compound laser system and fed into the sample by a dichroic beamsplitter. The anti-Stokes signal ($\omega_{AS}$) is generated in the sample. The forward signal (F-CARS) is collected by transmitted light detector (TLD). The backscattered signal (Epi-CARS) is collected by reflected light detector (RLD).

The colors indicate the relative wavelength only qualitatively. All electromagnetic components are infrared and therefore invisible. To ease visualization of the geometry, only peripheral rays are indicated. All beams are coaxial and cover the full aperture.
Dedicated CARS software
Despite the sophisticated technology, Leica TCS CARS is easy to handle and caters to the needs of beginners as well as those of the experts in the field. The multitude of possible adjustments, and the ease of setting up an experimental workflow, make it ideally suited for multi-user facilities.

Recall system settings with a single click
The control of Leica TCS CARS, which is an extension to the intuitive system software LAS AF, helps to comfortably set up the parameters for an experiment. Preferred system settings such as scan speed, image format, number of detectors, z-stacks, or time series can be stored and reloaded in Leica’s standardized Instrument Parameters Settings (IPS). This enables quick access to saved settings with a single click and helps with efficient utilization of microscope time.

Retain the overview with fully integrated software
The clearly structured user interface of Leica TCS CARS is designed for easy, ergonomic use. It guides the user through all necessary steps, always showing current options while hiding currently irrelevant ones. This applies to both the hardware and the software configuration.

“The integration of CARS technology into the Leica confocal platform means that label-free high-resolution microscopy becomes accessible for cell biologists.”

Prof. Andreas Zumbusch, Department of Chemistry, University of Konstanz, Germany
The control of the CARS settings is fully integrated in the user interface of the system software LAS AF.

Select the wave number according to the specimen.

Tune the laser to the wavelength needed either in the Raman mode or the pump mode and open the first laser safety shutter.

Select the NDDs either for F-CARS or Epi-CARS and open the second CARS laser safety shutter.
One interface fits all applications
Do you wish to perform experiments based on CARS, single photon, multiphoton fluorescence or with UV lasers? Do you prefer imaging at video rate or conventional scanning at high resolution? Do you wish to image in simultaneous or in sequential mode? Users can freely choose the laser source, the scanner model, and the imaging mode that optimally fits a specific application within the system software LAS AF. Leica TCS CARS ensures that experiments run smoothly, ergonomically, and intuitively.

Integrated database of vibrational modes
The database of vibrational energy that is integrated in the software provides a unified approach to save and recall the characteristic vibrational energy of various chemical bonds in the specimens. Every Leica TCS CARS system is equipped with a generic database that is populated with characteristic wave numbers of more than 25 common chemical bonds such as C=C, C-H, C=N, O-H, etc. Use this database to look up the chemical bond of interest and tune the laser to the corresponding wave number to obtain a chemical image of the specimen. Based on the research findings users may also modify, adapt, or expand the database to suit unique specimens.

Enter the wavelength in the pump-based mode or the wave number in the Raman-based mode

Pump-based laser tuning

Raman-based laser tuning

Expedite Your Work
Teaming with APE (Angewandte Physik & Elektronik GmbH) Berlin, Leica Microsystems has developed a compact CARS laser device, which is fully controlled by the system software LAS AF. The solution offers the most convenient way to perform CARS experiments for experts and novices alike.

The tight integration of the laser control in the software makes the complex process of adjusting multiple parameters inside the laser and in the CARS beam routing completely transparent. Users do not have to directly deal with setting up lasers for routine imaging sessions. The CARS laser is completely software controlled. For example, turning the laser on or off is simply done by checking or unchecking a check-box in the “Configuration” tab; tuning the laser to the desired wavelength or wave number comprises a single click of a button in LAS AF. The integrated CARS calculator determines the CARS wavelength that is currently being detected, and the software suggests the appropriate filter set for imaging. This makes CARS – which is generally a challenging third order nonlinear process applied by experts – achievable for everyone.

Maximum Transparency by Tight Integration of Leica TCS CARS

Skin of a mouse ear. The maximum projection shows the structure of the different layers. Signals arise from oil-covered hairs, lipids, cholesterols, and ceramids in the intercellular space of the corneocytes and from triglycerids and wax ester, which are located in the sebaceous glands.

Courtesy of Prof. Sunney Xie, Department of Chemistry and Chemical Biology, Harvard University, Cambridge, USA
Why choose between a high resolution morphology system and a real-time live cell imaging system when you can have the benefits of both in one system?

As fast and highly resolved as needed – Leica TCS CARS is a true confocal equipped with up to eight VIS laser lines and a CARS laser. It covers a wide range of scan speeds and imaging possibilities in a single system.

The new Leica TCS CARS comes with a tandem-scanning system mounted on a sliding device. This setup enables easy switching between conventional and resonant scanning. Whereas the conventional scanner serves for all morphology and classical speed live cell applications, the resonant scanner provides a wide range of high speed options. This flexible combination makes Leica TCS CARS an ideal device for multi-user facilities.
Deep Imaging With and Without Dyes

CARS and Multiphoton

Higher collection efficiency
In confocal microscopy optical sectioning is obtained by spatially filtering all emitted photons from layers that are not at the focal plane. The spatial filter constitutes a pinhole, which is located in an intermediate image plane.

Multi-photon techniques (such as CARS) intrinsically feature optical sectioning. These processes occur only at very high photon densities. The highest photon densities are found in the focal plane, which is the layer from which the CARS signal is detected.

As a pinhole is not needed in this case, it is possible to just collect as many scattered photons as possible without imaging optics. This is achieved with the non-descanned detectors.

Leica TCS CARS provides a four channel solution: two channels for Reflected Light Detection (RLDs) and two channels for Transmitted Light Detection (TLDs).

Tune the laser according to your needs
The Leica TCS CARS is equipped with two infrared sources in a single box: A Nd:Vanadate laser with a fixed wavelength of 1064 nm and an OPO (Optical Parametric Oscillator) that is tunable in a wavelength range from 780 nm up to 940 nm. This special setup offers CARS imaging and multiphoton imaging.

CARS laser offered by Leica Microsystems is directly coupled to a dedicated port in the scanhead of the confocal microscope and controlled by Leica LAS AF software. For precise attenuation of the laser, a continuously adjustable AOTF is integrated into the beam routing.
If your future research projects require CARS, the Leica TCS SP5 II can be upgraded to a fully equipped CARS system at any time. The upgrades are done on site by Leica Microsystems’ field service engineers.

Taking Service to a Higher Level

Less downtime with Leica RemoteCare
At Leica Microsystems we are committed to ensuring maximum uptime of your Leica confocal instruments. We offer a service concept that allows your system to be checked frequently and take measures before system downtime occurs.

The most important system parameters are recorded and evaluated to detect any malfunction before the system shows failure. With Leica RemoteCare we are prepared to help in time.

Leica customer service
We’re just a call away – wherever you are. Simply contact your local sales representatives to get superior engineering and application support or to contact your local sales and application specialists.

Operation and application training
We also provide in-depth training for getting the most out of your system and applications.

Maintenance contracts
Various levels of maintenance are available – from once-a-year minimum maintenance to our all-inclusive contract. Talk to your local service manager to find the best contract for you.
Leica TCS SP5 II Scan Head with CARS

1. Visible lasers (up to 5)
2. CARS laser
3. 405 nm CW laser
4. Visible range AOTF
5. CARS AOTF
6. 405 nm AOTF
7. 405 nm optics imaging
8. Acousto-Optical Beam Splitter (AOBS)
9. Switchable beam expander (optional)
10. Multi Function Port (MFP)
11. Tandem scanner
12. Calibration target
13. Field rotation optics
14. Objective lens
15. Reflected light NDD, Epi-CARS PMT
16. Reflected light NDD, Epi-SHG PMT
17. Transmitted light NDD, F-CARS PMT
18. Transmitted light NDD, F-SHG PMT
19. Confocal detection pinhole
20. Filter- and polarizer wheel with notch filter
21. X1-emission port
22. Spectrophotometer prism
23. PMT channel 1 (optional FLIM)
24. PMT channel 2
25. PMT channel 3
26. PMT channel 4
27. PMT channel 5 (optional FLIM)
The statement by Ernst Leitz in 1907, “with the user, for the user,” describes the fruitful collaboration with end users and driving force of innovation at Leica Microsystems. We have developed five brand values to live up to this tradition: Pioneering, High-end Quality, Team Spirit, Dedication to Science, and Continuous Improvement. For us, living up to these values means: Living up to Life.

Active worldwide

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Leica Microsystems operates globally in four divisions, where we rank with the market leaders.

- **Life Science Division**
  The Leica Microsystems Life Science Division supports the imaging needs of the scientific community with advanced innovation and technical expertise for the visualization, measurement, and analysis of microstructures. Our strong focus on understanding scientific applications puts Leica Microsystems’ customers at the leading edge of science.

- **Industry Division**
  The Leica Microsystems Industry Division’s focus is to support customers’ pursuit of the highest quality end result. Leica Microsystems provide the best and most innovative imaging systems to see, measure, and analyze the microstructures in routine and research industrial applications, materials science, quality control, forensic science investigation, and educational applications.

- **Biosystems Division**
  The Leica Microsystems Biosystems Division brings histopathology labs and researchers the highest-quality, most comprehensive product range. From patient to pathologist, the range includes the ideal product for each histology step and high-productivity workflow solutions for the entire lab. With complete histology systems featuring innovative automation and Novocastra™ reagents, Leica Microsystems creates better patient care through rapid turnaround, diagnostic confidence, and close customer collaboration.

- **Medical Division**
  The Leica Microsystems Medical Division’s focus is to partner with and support surgeons and their care of patients with the highest-quality, most innovative surgical microscope technology today and into the future.

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