Multimodal non-linear optical microscope system for CARS, SRS, SHG, TPEF, FLIM imaging and spectral analysis

Confotec CARS

CARS microscopy is a label-free imaging method that is capable of real-time, non-contact and nondestructive examination of various biological samples.

APPLICATIONS

- Biology Imaging
- Medical Diagnosis of Disease
- Material Science

MAIN SPESIFICATION

Epi-CARS and F-CARS Spatial XYZ resolution: < 0.7 µm
(Objective lens: 60x, NA=1.2, water immersion)
Spectral range:
- CARS scanning microscope (F-CARS & E-CARS): 500 – 4000 cm^{-1}
- Stimulated Raman scattering mode (F-SRS & E-SRS): 300-4200 cm^{-1}

Spectral resolution: 8 - 10 cm^{-1}
SHG Imaging spectral range: 400 – 516 nm
Scanner type: Galvanometer XY scanner (raster high-speed and start-stop mode)
Scanning speed: 1000x1000 points / 3 sec
Scanning range (fast scanning mode, 60x lens) XY: 225 x 225 µm, Z: 80 µm
Control and automation: Fully automated

Five independent high-speed channels:

1) F-CARS: CARS signal in forward direction
2) E-CARS & Raman: CARS signal in backward (EPI) direction/ Raman signal
3) SRS signal in forward direction
4) SRS signal in backward (EPI) direction
5) Reflected and transmitted signal detection
6) CCD spectrometer for Raman spectra detection

Excitation source: Tunable mono-block OPO laser system

Laser fundamental wave: > 0.7 W @ 1032 nm
OPO Signal: 700.. 900 nm, > 0.7 W @ 800 nm
Pulse width (fundamental and OPO output): 2 ps
Pulse repetition rate: 80 Mhz
Integrated EO Modulator (for SRS Imaging): 20 MHz

METHOD EXPLANATION

In the CARS technique, a pump beam at frequency ωp and a Stokes beam at frequency ωs interact with a sample. When the beat frequency ωp - ωs matches one of the vibrational modes in the sample, a strong anti-Stokes signal at ωas = 2ωp – ωs is generated in the phase matching direction (Fig.1).

Chemically selective information can be provided by tuning into characteristic vibrational resonances in samples.

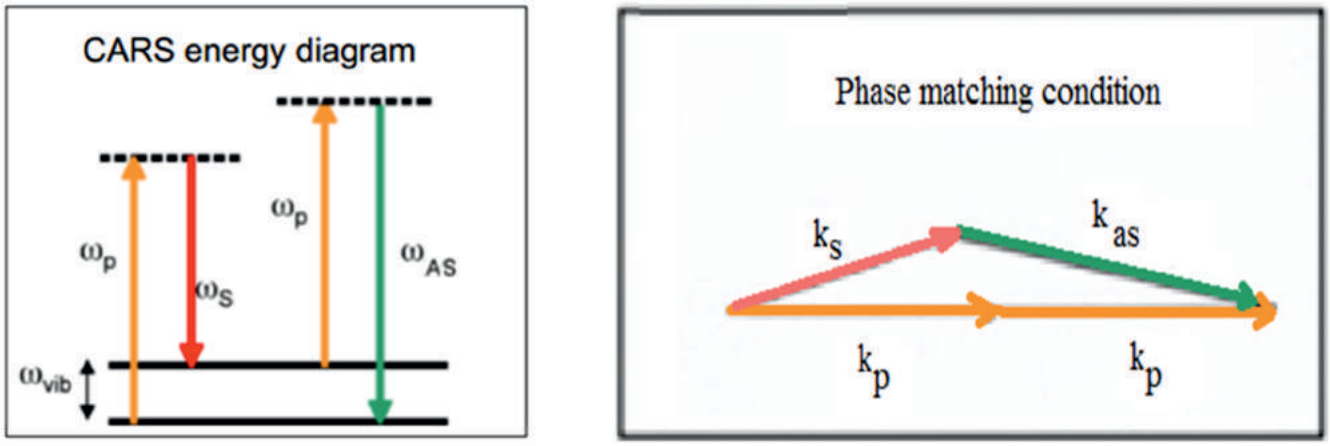


Figure 1. Energy diagram of CARS. ωp is a pump beam frequency, ωs is the Stokes beam frequency, ωas=2ωp-ω is the CARS signal at the anti-Stokes frequency. kp, ks and kas are corresponding wave vectors.

MAIN ADVANTAGES

The advantages of CARS technique are summarized as follows:

- CARS signals are 3-4 orders of magnitude stronger than those of spontaneous Raman process
- video-rate vibrational imaging at reasonable excitation powers
- sample photodamage is minimized due to usage of picosecond lasers
- the CARS signals are blue-shifted from the pump and Stokes frequencies, and they can be easily detected in the presence of any fluorescence background
- an enhanced spatial resolution, because the CARS signal is generated only from the focal volume, where two beams are overlapped (Stokes and pump beams)
- the capability of three-dimensional sectioning without the need of confocal geometry
- it does not require exogenous dyes or markers (any labeling may strongly affect the specimen properties)

CONFOTEC CAPABILITIES

The commercial CARS imaging microscope, Confotec CARS system (SOL instruments), is shown in Fig.2.

Figure 2. Confotec CARS system (SOL instruments)

The Confotec CARS imaging microscope allows a complete multimodal investigation of tissues, in particular:

- Coherent Anti Stokes Raman Microscopy (CARS), including F-CARS, E-CARS and P-CARS
- Stimulated Raman Scattering (SRS) Microscopy
- Raman confocal imaging
- Fluorescence Life Time Imaging (FLIM) Microscopy (Option), including FRET and FRAP
- Two-Photon Excited Fluorescence (TPFE) Microscopy
- Second-harmonic Imaging (SHG) Microscopy
- Laser reflection & transmission imaging
- Upconversion Luminescence
- SONICC imaging (SONICC is an emerging technique for crystal imaging based on second harmonic generation effect found in chiral crystals)

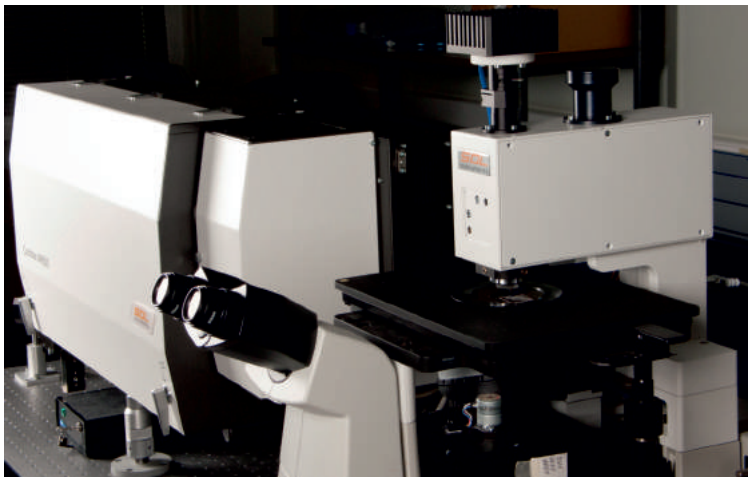


IMAGE EXAMPLES

The following examples are provided to demonstrate the quality of results.

Figure 3. CARS/TPEF imaging of cancer HeLa cells. Ptoteins (blue color) and lipids (grey color) have been observed in the CARS mode at their characteristic vibrations of 2930 cm^{-1} and 2840 cm^{-1}, respectively. Nucleic acids, strained by acridine orange, have been acquired in the red (Ribonucleic acid, RNA) and green (DNA) fluorescence channels in TPEF mode. *(Courtesy of A.Kachynski, A. Kuzmin, and P. N. Prasad, The State University of New York at Buffalo, eqiupment: Confotec CARS)*

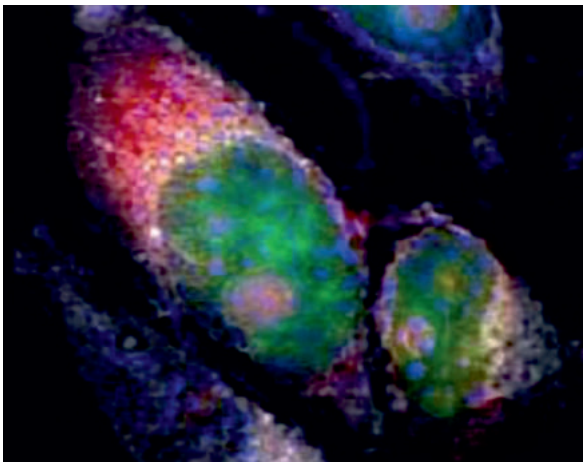


Figure 3.

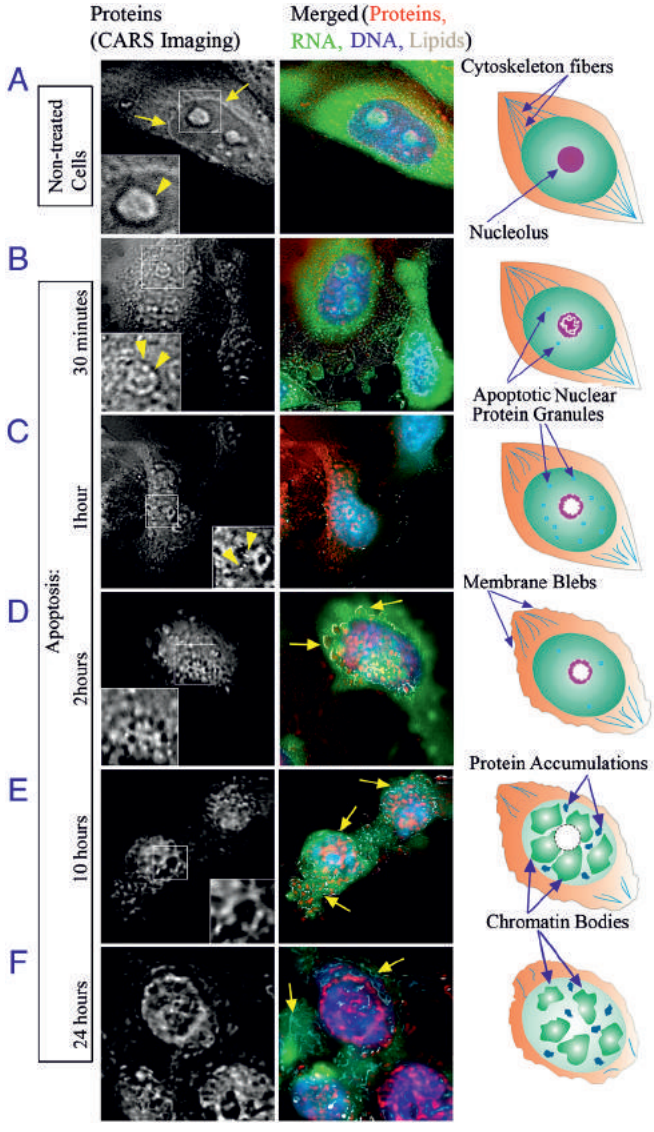


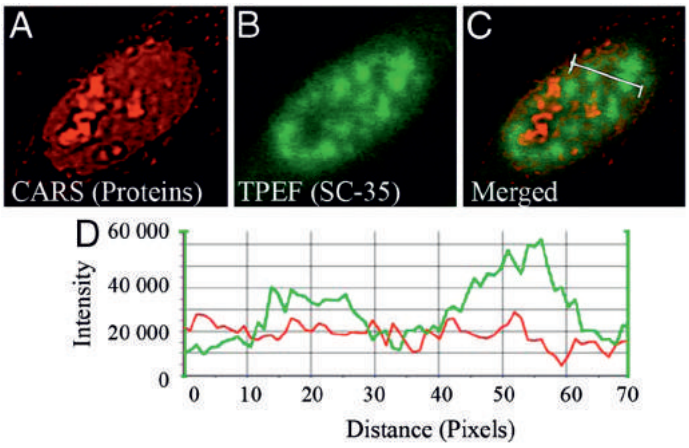
Figure 4.

Figure 4. The distribution of proteins, lipids, DNA, and RNA in dividing and apoptotic HeLa cells visualized by multimodal CARS/TPEF imaging. During the imaging live cells were maintained at the physiological conditions. Proteins and lipids were observed in the CARS mode at their characteristic vibrations of 2930 cm-1 and 2840 cm-1, respectively. Nucleic acids, stained by acridine orange, were acquired in the red (RNA) and green (DNA) fluorescence channels in TPEF mode. In the right panels, schematics of the macromolecular organization of cells are represented. The CARS signal from proteins is represented in the left panels. The panels in the middle represent merger signals of the proteins (red), RNA (green), DNA (blue),and lipids (gray). The white-outlined areas in the protein channel are enlarged below. (A) Nontreated cells. The signal from proteins is accumulated in the nucleolus (Inset, arrowhead) and the nuclear lamina (arrows). In the rest of the nuclear volume, the intensity of the protein signal is nearly uniform. (B–F) Representative cells at the subsequent stages of the apoptotic development. (B) 30 minutes following the initiation of apoptosis, the distribution of proteins is altered in the nucleolus (Inset, arrowheads) and the novel structure, apoptotic nuclear protein granules (ANPG), emerged in the nucleoplasm. In the cytoplasm, cytoskeleton fibers begin to lose their structural polarization. (C) The pattern of proteins becomes increasingly irregular, and ANPG become prominent (Inset, arrowheads). (D) The nucleolar proteins are forming a complex meshwork (Inset).The apoptotic membrane blebs are seen (D and E merged panels, arrows), ANPG disintegrate. (E and F) Proteins abandon the nucleolus and demonstrate a highly irregular distribution in the nucleoplasm; the genomic DNA is condensing to chromatin bodies and partially segregates from the proteins. *(Artem Pliss, Andrey N. Kuzmin, Aliaksandr V. Kachynski, and Paras N. Prasad PNAS July 20, 2010. 107 (29) 12771-12776, eqiupment: Confotec CARS)*

IMAGE EXAMPLES

Figure 5. Simultaneous visualization of the bulk nuclear proteins in CARS mode and specific protein epitopes (SC-35) in the TPEF mode. Imaging was performed in the fixed cells. (A) Proteins were visualized at characteristic vibration of 2930 cm-1 in the CARS mode. (B) Marker of the nuclear speckles, SC-35 was labeled with fluorochrome-conjugated antibodies and visualized in the TPEF mode simultaneously with the proteins CARS signal. (C) A merged image of A and B. (D) An intensity line profile of SC- 35 (green line) and proteins CARS signal (red line) drawn through C. The intensity of CARS signal is relatively uniform in the nuclear speckles and in the surrounding nucleoplasm. *(Artem Pliss, Andrey N. Kuzmin, Aliaksandr V. Kachynski, and Paras N. Prasad PNAS July 20, 2010. 107 (29) 12771-12776, eqiupment: Confotec CARS)*

Figure 5.



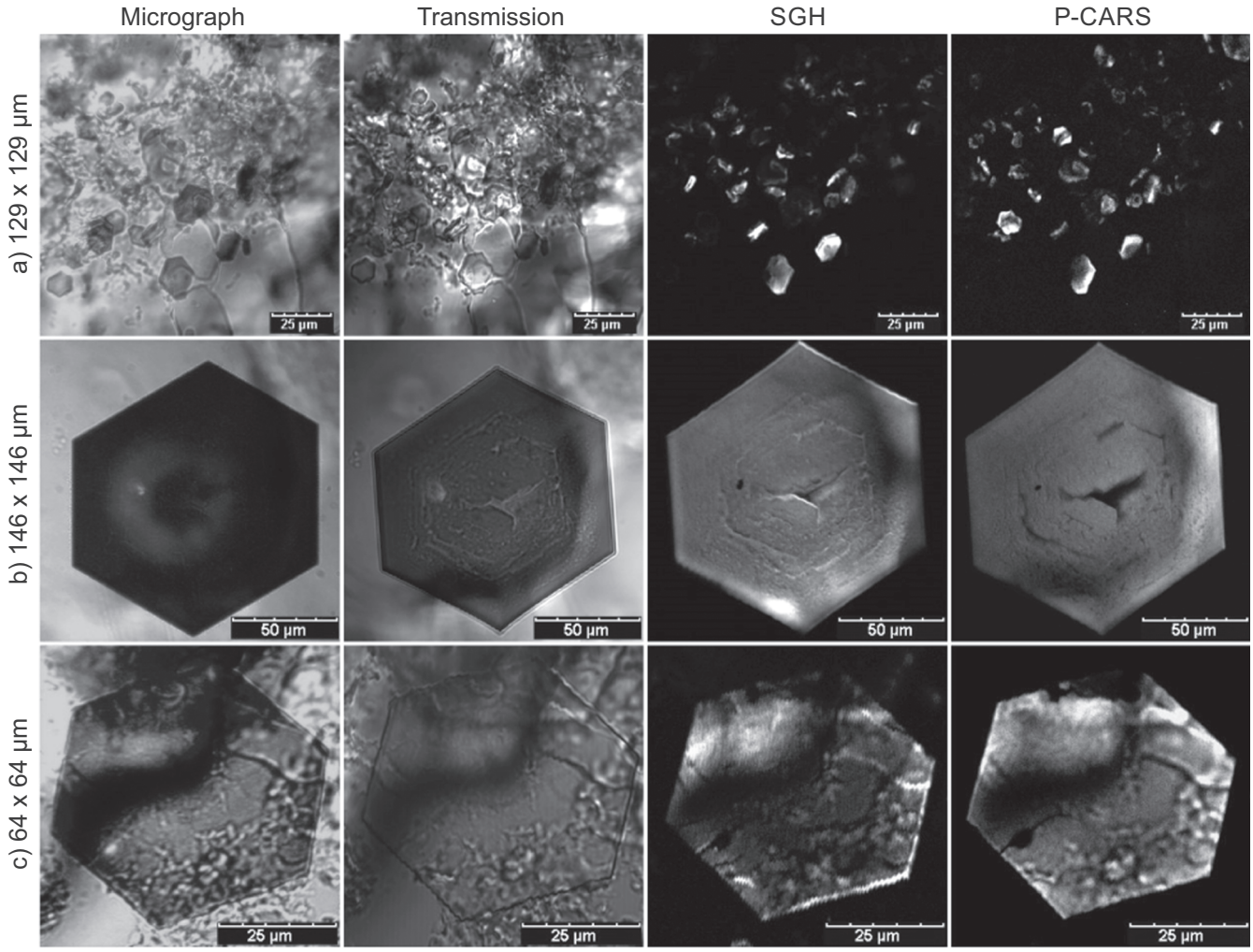


Figure 6. Imaging of bacteriorhodopsin (BR) crystals. *(Arzumanyan, Grigory M. J. Am. Chem. Soc., 2016, 138 (41), p. 13457, eqiupment: Confotec CARS)*

SUMMARY

Confotec CARS system represents a novel and sensitive tool for biology and materials sciences. The experimental data demonstrate fast and nondestructive technique of the CARS imaging approach.