

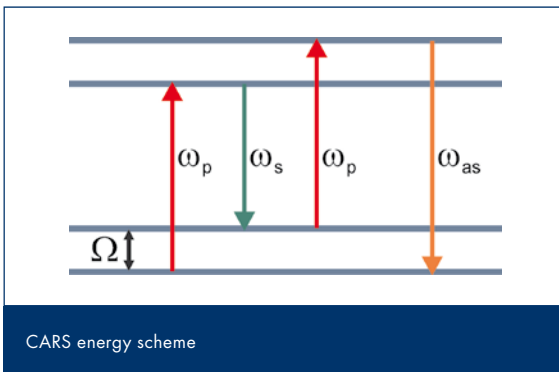
Application Note

CARS microscopy

In the last ten years multi-photon-microscopy has become increasingly popular to enhancing contrast and resolution for modern microscopic imaging due to the utilization of non-linear optical effects. Amongst many methods of multi-photon-microscopy, two-photon-excitation fluorescence-microscopy is the most popular one with more than a few hundred systems installed every year.

The big draw back of two-photon-fluorescence microscopy, however, is the use of dyes for creating the images. Methods to overcome the problems connected with dyes are based on non-linear optical effects such as second and third harmonic generation (SHG, THG), Raman scattering or Coherent Antistokes Raman Scattering (CARS), a third order non-linear process ($\chi^{(3)}$ process) involving four photons.

Coherent Antistokes Raman Scattering



In CARS, a pump field at the frequency ω_p and a Stokes field at ω_s hit the sample and induce vibrations of molecules at the beat frequency $\omega_p - \omega_s$. The excitation of these vibrations is most effective if the difference frequency $\omega_p - \omega_s$ equals the characteristic Raman frequency Ω of the molecule. The anti-Stokes side band at the frequency $\omega_{as} = (\omega_p - \omega_s) + \omega_p$ (see figure on the left side), the signal which is finally measured, is generated by the above mentioned cubic nonlinear process. With the actively driven oscillations and the coherent signal generation it is possible to achieve much stronger signals with CARS as it is the case with conventional Raman scattering. The utilization of CARS for microscopic imaging is creating contrast without dyes and is yielding a strong spectrally blue shifted signal ω_{as} , which can be easily detected and discriminated from the excitation fluorescence background.

CARS microscopy images

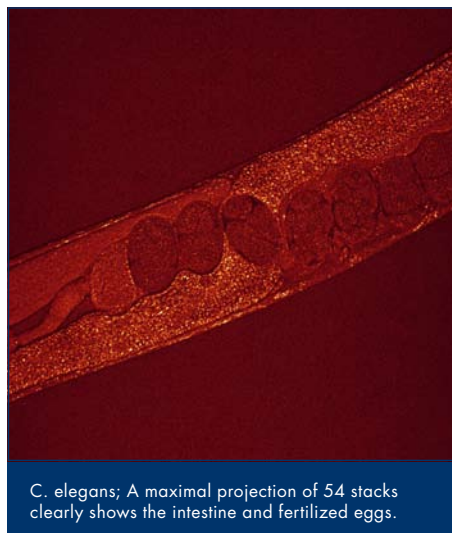
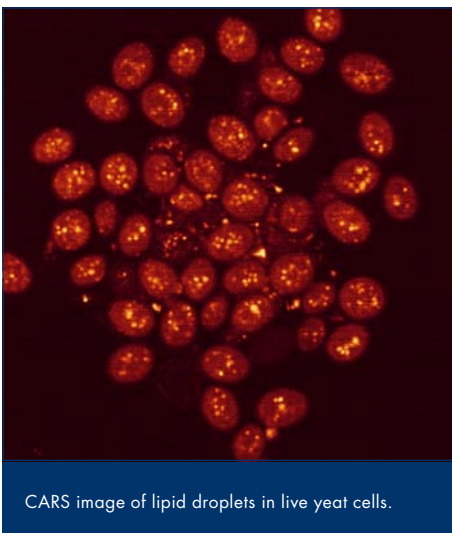
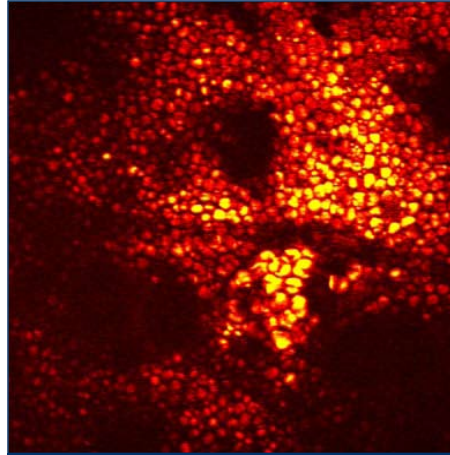


Image courtesy:
M. Winterhalder, A. Zumbusch
University Konstanz

CARS microscopy images



CARS image of individual *Bacillus subtilis* bacteria, measuring 1 micron by 5 microns in size.



FM-CARS image of adipocytes in a white mouse ear showing lipid-rich contrast.

Image courtesy:
Prof. Sunney Xie
Harvard University

picoEMERALD - CARS light source



picoEMERALD
APE powered by High Q Laser

The **picoEMERALD** comprises a high power picosecond oscillator (**picoTRAIN™**) with a frequency doubler and a synchronously pumped OPO in a single housing. The optical modules were optimized by finite element analysis and mechanical stability algorithms (misalignment stability optimization) to exhibit maximum passive stability. In addition an active resonator control is maximizing continuously the efficiency of the high power picosecond oscillator and the OPO. The **picoEMERALD** supplies three temporally and spatially overlapping ultrafast pulse trains: 1064 nm out of the laser oscillator itself and 690 nm ... 990 nm (Signal range) and 1150 nm ... 2300 nm (Idler range) from the OPO. The wavelength tuning occurs automatically by PC. Due to the use of a synchronously pumped OPO pumped by a modelocked solid state laser the pulses show no timing jitter and a very low noise level. Best spectroscopic resolution requires a picosecond excitation with a typical bandwidth of $\sim 10 \text{ cm}^{-1}$. The **picoEMERALD** delivers 7 ps at 1064 and 5-6 ps from the OPO to match this requirement. Independent power adjustments of the 1064 nm beam and the OPO Signal and Idler, sensors for the spatial and temporal overlap and a high resolution spectrometer are included. 1064

nm pulses and OPO Idler pulses can be independently combined with the OPO Signal or blocked. The standard CARS set up is utilizing the 1064 nm as Stokes and the Signal pulse train as Pump with an energy difference from 700 to 5000 cm^{-1} . Other CARS set-ups are using the Signal and Idler pulse trains (1400 to 10.000 cm^{-1}) for higher penetration depths due to the red shifted excitation wavelengths. The specified 750 mW for both 1064 nm and the OPO-Signal output as well as 600 mW for the OPO-Idler provide more than enough power for CARS and coherent Raman microscopy.



High Q Laser inside: **picoTRAIN™ GREEN**