

Multiplex CARS Microscopy

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Introduction

The combination of Multiplex Coherent Anti-Stokes Raman Spectroscopy (MCARS) with microscopy presents a non invasive and labeling free technique and therefore embodies a powerful nonlinear method for the study of multiple problems in material and biological science. [1-2] The MCARS-microscope takes full vibrational spectra for each position in the sample within tens of milliseconds making MCARS in particular interesting for observing spatially resolved chemical and biological changes. In general, such broadband-CARS describes the possibility to generate a Raman-spectrum that covers a spectral range of more than 3000 wave-numbers (Fig. 1).

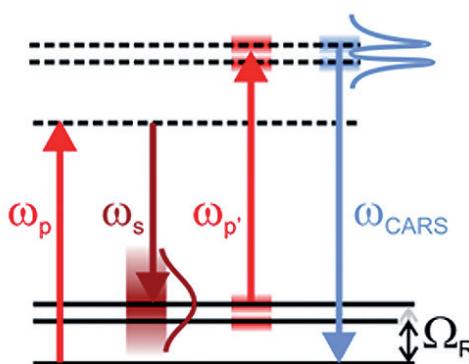


Fig. 1. Schematic depiction of a multiplex CARS process. Instead of one frequency ω_s , a broader spectrum is used that allows excitation of several vibrational levels at once. The coherently excited vibrational levels are probed by ω_s to generate the CARS signal at $\omega_{\text{CARS}} = \omega_p + \Omega_R$. This leads to a blue-shifted CARS signal which mirrors the vibrational spectrum. (Image from Ref.[1])

Experiment

Our experimental MCARS setup (Fig. 2) used the emission (1.0 W) at 800 nm of a Ti:Sapphire oscillator (Coherent Mira 900 pumped by Coherent Verdi V10) with a repetition rate of 80 MHz.[3] Filter F1 is a narrowband pass filter where most of the light is reflected. Only a narrow spectrum at the center wavelength is transmitted and acts as pump and probe. A fraction of the initial beam (100 mW) is used to create a supercontinuum in an end-sealed PCF (type NL-PM-750, crystal fiber A/S) and is used as Stokes pulse, resulting in a spectral range of more than 3500 cm⁻¹ supported. MO1 in Fig.2 represents a commercially available phase contrast microscope (Olympus BX 51) modified for MCARS application. The signal detection occurs by a spectrograph (Acton SP2300) with a sensitive EMCCD (NEWTON DU970N-BV model).

Application Note

At each point of the raster scanned sample (100×100 pixels, step size = 1 μm), a complete MCARS spectrum can be detected with an acquisition time as fast as 5-50ms, depending on signal intensity.

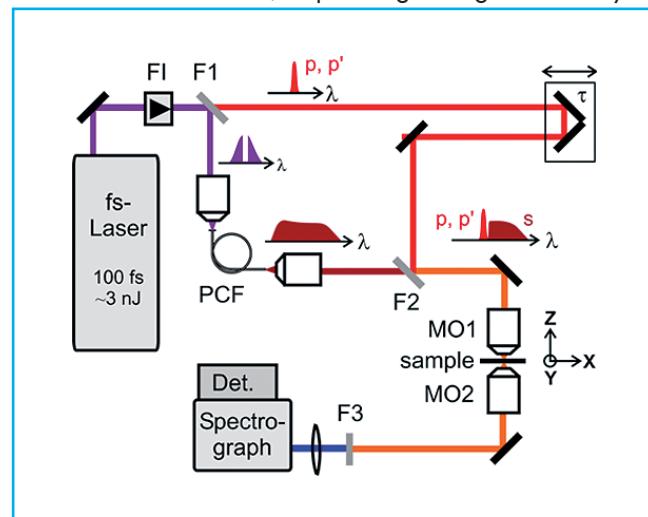


Fig. 2. Schematic drawing of the experimental broadband MCARS setup. FI: Faraday isolator, F1: bandpass interference filter, PCF: Photonic crystal fiber, F2: longpass interference filter, MO1: microscope objective (60x, NA 0.7), MO2: microscope objective (40x, NA 0.6), F3: shortpass interference filter.

Results

An important aspect in MCARS microscopy is the identification of weak signals. In this regard, the signal-to-noise (SNR) ratio plays an important role. Figure 3 shows a comparison of a MCARS measurement of a cellulose derivative using two different detectors. The normal CCD detector (Fig.2(a)) detects clearly the C-H spectral features at around 2800 cm⁻¹, but the C=C mode cannot be distinguished from the noise. Contrasting to that, the enhanced mode of the Newton EMCCD allows us to obtain a similar signal amplitude but with an improved SNR (Fig. 2(b)).

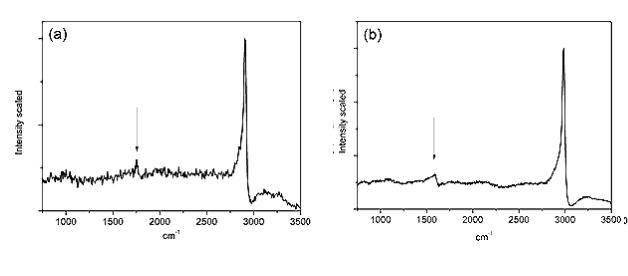


Fig. 3. MCARS spectrum of a cellulose derivative with a (a) non EMCCD-based detector compared to a (b) Newton EMCCD DU970N-BV from Andor Technology.

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Application Note

The capability of broadband detection is very important, when the composition and therefore the Raman-spectroscopic behavior of the sample are completely unknown. Especially for biological tissues, the C-H-stretching vibrations are often the strongest signal that can be detected and the line shape as well as the interplay with nonresonant background have to be taken into account for data analysis. An example of MCARS imaging is show in Fig. 4 for a biological sample.^[3] The reconstructed Raman spectra of different biological components were used to resolve the cellular structure. In green, the intercellular area is shown which is rich in cellulose. The structures in red mark the chloroplasts and the associated chlorophyll. The region in blue lacks a Raman spectrum and is related to vacuoles.

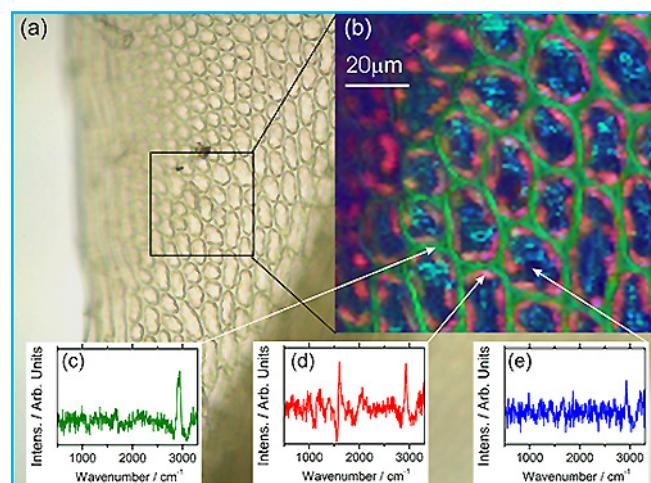


Fig.4: (a) Brightfield image of cells of *Plagiomnium rostratum*. (b) A MCARS image of the selected region in (a), after principal component analysis (PCA). The reconstructed Raman spectra of three biological components are shown in (c)-(e). (Image from Ref.[(3)])

References

- [1] B. von Vacano, L. Meyer, and M. Motzkus, "Rapid polymer blend imaging with quantitative broadband multiplex CARS microscopy," *Journal of Raman Spectroscopy* 38, 916-926 (2007).
- [2] J. X. Cheng and X. S. Xie, "Coherent anti-Stokes Raman scattering microscopy: Instrumentation, theory, and applications," *Journal of Physical Chemistry B* 108, 827-840 (2004).
- [3] C. Pohling, T. Buckup, and M. Motzkus, "Hyper-spectral data processing for chemoselective multiplex coherent anti-Stokes Raman scattering microscopy of unknown samples," *Journal of Biomedical Optics* 16, 021105 (2011).

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