

QUANTITATIVE SPECTRAL ANALYSIS IN MULTIPLEX CARS MICROSPECTROSCOPY

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In contrast to conventional Raman scattering microscopy, the contrast mechanism in coherent anti-Stokes Raman scattering (CARS) microscopy is based on the coherent and nonlinear interaction of a pump and a Stokes laser pulse with the sample, in which the molecular bonds oscillate in phase and spectrally interfere with each other as well as with the nonresonant CARS contribution of the sample. This coherent nature of CARS prevents the direct quantitative analysis of CARS spectra, i.e. without the retrieval of hidden phase information the direct determination of the concentration of the individual chemical components of the sample is not feasible.

Here, we report on the evaluation and comparison of different approaches to extract the full quantitative information from multiplex CARS spectra recorded for a variety of microscopic samples of heterogeneous chemical compositions:

1. Least-square fitting of the CARS spectrum, where the vibrationally resonant contribution to the third-order susceptibility is modeled as a sum of complex Lorentzian Raman lines, allows the reconstitution of the linear Raman response in a quantitative manner [1-3]. This approach requires a detailed *a priori* knowledge of the vibrational resonances involved, which are typically obtained from the spontaneous Raman scattering spectral properties of the individual chemical components. This procedure has been successfully applied for the spectral analysis of CARS spectra from a mixture of lipids that constitute the *stratum corneum* of skin tissue.
2. In the limiting case, where a very weak resonant CARS signal is overwhelmed by an intense nonresonant CARS signal, the recorded CARS spectra are directly proportional to the sum of real parts of the Raman resonant contributions to the third-order susceptibility [4]. This in turn allows a simple and model-free analysis of the data, which will be exemplified by the quantitative spectral analysis of CARS spectra obtained from a single protein fibril.
3. An elegant alternative approach for the spectral analysis of recorded CARS power spectra makes use of the maximum entropy (ME) method [5,6], which has the advantage that no *a priori* information regarding the Raman resonances of the sample is required. The ME method has therefore been applied for the extraction of chemical composition and Raman response from CARS spectra of intracellular components and inside tissue. Results obtained by the ME approach will be compared with those obtained from a decomposition analysis of CARS spectra.

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