SPECTRAL UNMIXING OF HETERODYNE CARS MICROSCOPY IN THE COMPLEX PLANE

Erik T. Garbacik¹, Jeroen P. Korterik¹, Cees Otto², Alina Zare³, Jennifer L. Herek¹, and Herman L. Offerhaus¹

¹ Optical Sciences group, MESA+ Institute for Nanotechnology, Faculty of Science and Technology, University of Twente, PO Box 217, 7500AE Enschede, The Netherlands
² Medical Cell BioPhysics group, MIRA Institute of Technical Medicine and Biomedical Technology, Faculty of Science and Technology, University of Twente, PO Box 217, 7500AE Enschede, The Netherlands
³ Electrical and Computer Engineering Department, University of Missouri, 327 Engineering Building West, Columbia, MO 65211, United States of America

Email: h.L.offerhaus@utwente.nl

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Determining the quantities, distributions, identities, and sizes of the constituent particles in mixtures is of significant interest, particularly in dynamic systems. Imaging modalities where contrast is derived from molecular vibrations are strong candidates for fulfilling this role. In particular, coherent anti-Stokes Raman scattering (CARS) fulfills all of the appropriate criteria: chemically-specific imaging can be performed up to video rate on samples with little or no additional preparation. A significant drawback is that CARS data is inherently non-quantitative due to a nonlinear mixing of the resonant signal of interest and a persistent, frequency-independent non-resonant background.

However, as CARS is a coherent process, both the amplitude and phase of the nonlinear response can be measured using heterodyne methods. We have extended one such technique, known as vibrational phase contrast (VPC)-CARS [1,2], with hyperspectral capabilities, enabling the acquisition of the amplitude and phase of the nonlinear molecular response at every spatial location within a sample over a range of closely-spaced vibrational frequencies. We have used this technique to image complicated mixtures of compounds, and applied a sparsity-promoting iterative constrained endmember (SPICE) [3] algorithm that explicitly utilizes the full complex space of the vibrational information to extract the pure underlying vibrational spectra. With this combined system we can determine the proportions of the pure compounds at every pixel in the sample.