Shot-noise limited detection sensitivity in multiplex CARS microscopy of single lipid bilayers

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Coherent anti-Stokes Raman scattering (CARS) microscopy has been shown to be a unique tool to visualise both chemical composition and structural features in a variety of specimens without the use of labels, in three dimensions and with optical resolution. In this presentation we demonstrate that multiplex CARS microscopy can provide the detection sensitivity to monitor structural features in samples that consist of only a single molecular layer.

There are two different approaches to optimise the signal detection sensitivity in CARS microscopy: (i) minimising the non-resonant background or (ii) detecting the full signal in multiplex mode. In the first approach, the sensitivity issues related to the non-resonant background, are circumvented by reducing its contribution as much as possible either through polarisation schemes, separation in the time domain or utilising the properties of the CARS signal generated in the backward (epi) direction. In this presentation we demonstrate an alternative approach to high sensitivity CARS microscopy: detection of the complete - resonant and non-resonant - CARS signal in a multiplex mode, where the anti-Stokes signal is generated simultaneously over a significant part of the vibrational spectrum. We show that in multiplex CARS the signal-to-noise ratio of the measurement is independent of laser related fluctuations (e.g. power, pulse width, jitter) but limited only by the inherent Poisson noise. The availability of the spectral information is also crucial for the identification of the structural state of the sample or to identify minority chemical species.

We present experimental results on sensitivity studies of the multiplex CARS technique and direct measurements of the thermodynamic state of single lipid mono- and bilayers based on the intrinsic vibrational properties of the molecules (figure 1). Using CARS microscopy with shot-noise limited sensitivity, spatially resolved vibrational spectra of **single** lipid bilayers are acquired with a signal-to-noise ratio of 4 in 20 ms. From these spectra the lipid chain order for both liquid-crystalline and gel phase supported lipid bilayers can be determined directly.

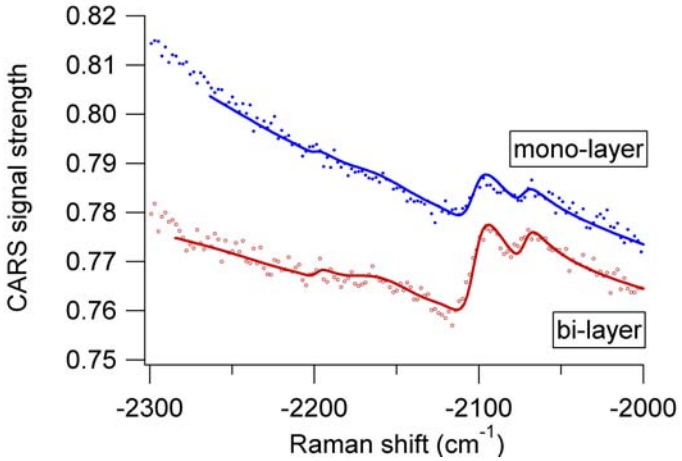

Figure 1. Multiplex CARS spectra of a single leaflet (upper curve) and single lipid bilayer (lower curve). Using these spectra lipid chain order parameters can be determined.