Raman microscopy for high resolution chemical imaging in living cells

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Raman microspectroscopic techniques, such as spontaneous Raman microscopy (spRm) and coherent anti-Stokes Raman microscopy (CARM), presently represent the most informative optical methods for high resolution, label-free imaging of the chemical state. It is well-known that the particular strength of the Raman techniques is derived from the vibration-modulated polarizability. Although the Raman cross section and the CARS cross section are much smaller than the cross section associated with dipolar transitions in fluorescent molecules, these can be probed with high accuracy with ultra-sensitive spectrometers and detection methods. The spectroscopic information in spRm and CARM is quite similar, however the coherent nature of the signal generation in CARM as opposed to the spontaneous emission in spRm makes that both methods offer different opportunities. Particularly in CARM the continuous improvement in state-of-the-art of laser technology is important. Our approach is to use an optical parametric oscillator (OPO) as the source for synchronized, short light pulses. The OPO offers a variety of beams that can all be used in several approaches to CARM and, as we will show, can also be used in high resolution coherent anti-Stokes Raman spectroscopy (CARS). We have recently integrated a green-pumped OPO, with lithium triborate (LBO) as the active element, with a CARM set up[1]. Features of this microscope will be discussed in relation to several issues like hyperspectral CARS imaging, tunability and stability. The opportunities, that this system offers, to enhance the detectability of low concentration molecular species by heterodyne CARS will also be exemplified and discussed.
The extremely broad bandwidth and high spectral resolution imaging in Raman spectroscopy gives unprecedented insight in the chemical composition of a sample. The complementarity of the spRm and CARM approaches will be highlighted and examples of spRm on live cells will be presented.

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