Epi-detected multiplex CARS of cells and biomaterials

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Raman microscopy is rapidly developing as a powerful technique for biomaterial imaging in cells and tissues[1,2]. Spontaneous Raman microscopy is at the moment probably the most advanced and insightful because of its inherent multiplex properties. Coherent anti-Stokes Raman scattering microscopy is in a number of technological aspects complementary to the spontaneous variant. Particularly the signal strength in CARS can be many orders of magnitude higher than in spontaneous Raman scattering. This is a result of the stimulated population of (ground state) vibrations and the coherent nature of the scattering process. The high photon flux in the CARS process enables rapid imaging times and/or large fields of view. Together with the chemical specificity of the information content makes CARS and its variants a very important tool in material science, including biomaterials [3].
The third-order susceptibility contains non-vibration resonant contributions, electronic resonance contributions next to the desired vibration resonant contributions and it is not always evident what generates the contrast in images obtained by CARS microscopy. The use of multiplex - or broad-band CARS enables however a direct insight in the preponderance of one or another term in the susceptibility. This is especially the case when the so-called polarization sensitive CARS variants are used.
The theory of CARS is well known [3] and the spectral aspects can be summarized in fairly straightforward formulas. The beauty of the description lies in the fact that a comprehensive description of the spectral fingerprint is available. A global fit procedure enables a quantitative assignment of all components in the third-order susceptibility[4]. Furthermore we will show the first epi-detected broad band CARS images. On the basis of the multiplex approach and global fitting analysis an unequivocal assignment of the signal to the relevant chemical information can be given.

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