RAMAN IMAGING COMBINED WITH 2-PHOTON EXCITED FLUORESCENCE IMAGING

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A number of advances in Raman microscopy will be reported. We will show: 1) that Raman microscopy can be successfully combined with 2 photon excited fluorescence imaging, 2) that the Raman imaging time can be 5-fold decreased to a few minutes when lipid distributions, for instance in single cells, are imaged, 3) that the analysis of the high – and low spectral regions in Raman imaging can be exploited to discern lipid – rich areas in individual cell.

Raman microscopy is advancing steadily as a powerful technique for biomaterial imaging in cells and tissues[1,2]. The low photon flux in spontaneous Raman scattering requires long measurement times, typically of 1 second [1], per pixel. Large fields of view can therefore only be imaged from objects that are very stable over time. It is not always clear which is the area of interest for high-resolution chemical analysis. We have successfully investigated [3] the possibility to combine two-photon excited (2PE) fluorescence microscopy with Raman microscopy. A common near-UV molecular marker for DNA, Hoechst 33342, can be 2PE with 647.1 nm and has a Stokes emission that is spectrally positioned on the anti-Stokes side of the same laser line that is used to excite the Raman spectrum. This result makes it possible to use 2PE fluorescence imaging as a guide to areas from which Raman spectra need to be acquired.

The chemical imaging capabilities of spontaneous Raman microscopy principally relies on the inherent broad-band spectral information, which can be recorded with great accuracy. The spectral resolution in a typical Raman spectrometer [1] measures the spectral information with > 2 cm^{-1} resolution. This gives ample opportunity to distinguish the fingerprint spectra of many different chemical compounds, although the Raman spectra are crowded. The complicated chemical composition of cells and tissues, particularly, lead to strongly overlapping bands. Although the fingerprint spectral range is most informative, the high frequency spectral range is very useful as the Raman light scattering of lipid molecules is intense and readily discernible in spontaneous Raman spectra. The high Raman scattering cross section of the lipid compounds makes it possible to speed up Raman imaging significantly, which leads to unprecedented Raman imaging speeds at a confocal resolution. An analysis of lipid composition and structure and the spatial distribution in single cells will be presented.

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