BESSEL BEAM CARS MICROSCOPY FOR HIGH THROUGHPUT LABEL-FREE QUANTITATIVE CHEMICAL IMAGING

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Vibrational micro-spectroscopy is a powerful technique in cell imaging, providing chemical specificity without the need for exogenous labels. In the last decade, coherent Raman techniques (such as coherent anti-Stokes Raman Scattering, CARS) have been developed, complementing spontaneous Raman with the benefit of faster acquisition speed compatible with life cell imaging. However, high chemical specificity can be obtained only if the vibrational spectrum is measured for each spatial point. The resulting acquisition speed for hyperspectral volumetric imaging is not compatible with high throughput measurements.
Here we show how to supersede this limitation. Firstly, we have developed a Bessel beam excitation, which provides a "self-healing" extended axial point-spread function while not compromising the lateral resolution, allowing to use a single 2D acquisition to detect the volumetric chemical composition in 2D cell cultures of 5-10μm height. Introducing an axicon – lens combination in the excitation beam we obtain a chemical concentration point spread function that has an axial full-width-at-half-maximum twice as long as the Gaussian beam (see Figure a-d). The distribution of chemical components from FSC^3 [1] obtained by analysing 2D images acquired with the Bessel beam are equivalent to the results obtained by averaging along the axial stack of 2D images measured with the Gaussian beam (see Figure e-f). Secondly, we have reduced the amount of spectral points required by implementing a reconstruction algorithm based on sparse sampling without losing chemical specificity [2,3]. We find that the statistical analysis of the chemical concentration maps obtained with the sparse sampling approach can provide similar information as the maps obtained with full spectral resolution. The combination of Bessel beam excitation and sparse sampling results in a two orders of magnitude reduction of acquisition time, paving the way to the application of CARS micro-spectroscopy for high-throughput label-free chemical analysis of biological specimens, as we will show on a proof-of-principle study on drug induced phospholipidosis.

[1] Anal. Chem. 85, 10820 (2013). [2] Opt. Express 22, 4021 (2014). [3] J. Raman Spectrosc. 46, 727 (2015).

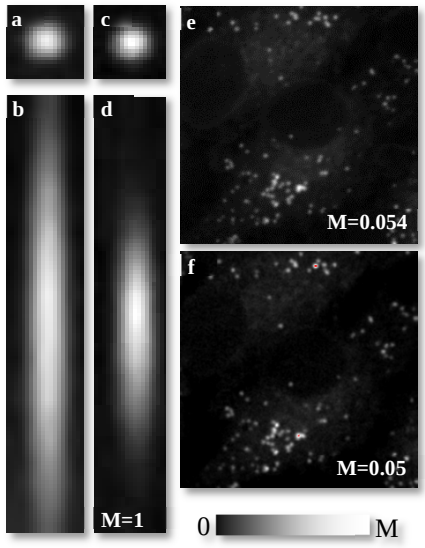

Figure: a,c) *xy* and b,d) *xz* concentration maps of the polystyrene component obtained from analysing 3D hyperspectral CARS images of a polystyrene bead (nominally 1μm in diameter) using a Bessel beam (a,b) or a Gaussian beam (c,d). The scale bar shows 2μm. The full-widths-at-half-maximum of the concentration profiles are 1.0μm (0.9μm) in the *x* direction for the Bessel (Gaussian) beam excitation, and 10.1μm (4.7μm) in the *z* direction. e,f) Concentration maps of the dry lipid component obtained by analysing hyperspectral CARS images of fixed HeLa cells using a Gaussian (e) or a Bessel (f) beam. The 2D image in e) has been obtained by averaging the 3D images weighted by the Bessel beam point spread function. Scale bar 10μm.