CARS imaging in the fingerprint region with a fs Laser and frequency-doubled OPO

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Label-free imaging with coherent anti-Stokes Raman scattering (CARS) proves to be a useful complimentary tool to fluorescence microscopy for easy visualisation of lipids, proteins and other biomolecules [1]. Being a non-linear, multiphoton imaging technique, CARS requires sophisticated pulsed laser systems to deliver high peak powers at two different excitation wavelengths. Two-photon fluorescence (TPE) and second harmonic generation (SHG) microscopy are two of the more common multiphoton techniques used in bio-imaging. Microscopy setups with these techniques almost always use a widely tuneable Titanium doped Sapphire laser (Ti:Sa) in combination with a long wavelength tuneable optical parametric oscillator (OPO) and suitable laser equipment is available in many research groups and biological imaging facilities [2]. The implementation of CARS microscopy on these systems is possible although not yet prevalent. Furthermore, it is only feasible to reach the Raman active CH_2 stretching region from 2700 cm^{-1} and higher due to the inherent energy gap between laser and OPO signal wavelengths [3, 4]. In this work we describe a straightforward approach to reach the fingerprint and Raman silent region for CARS with the same setup using a simple frequency-doubling modification. We show that CARS imaging in cells and tissues at 1550 cm^{-1} and 2100 cm^{-1} becomes accessible. This makes CARS imaging much more versatile on such systems.

For medical applications, we demonstrate that with our system, CARS can be employed to scrutinise the activity of alveolar macrophages. This could be useful for monitoring respiratory disease therapy, particularly in infants as the macrophages are easily accessible through lavage. The advantage of CARS is that these cells can be imaged straight away without staining and in high quality. We show that CARS can be used to investigate the pulmonary surfactant feedback mechanism. Future experiments will show the dependence of this mechanism on surfactant proteins such as SP-A and SP-D.

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