

Optical parametric oscillator system for coherent anti-Stokes Raman scattering microscopy of lipid prevalence and aggregation in fibroblast cells

W. Zhang¹, M. Parsons² and G. McConnell¹

¹Centre for Biophotonics, SIPBS, University of Strathclyde, 27 Taylor Street, Glasgow, G4 0NR, United Kingdom

²Randall Division of Cell and Molecular Biophysics, King's College London, Guy's Campus, London, SE1 1UL, United Kingdom

Corresponding author email: g.mcconnell@strath.ac.uk

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Coherent anti-Stokes Raman scattering (CARS) imaging as a label-free nonlinear microscopy method has proven successful in a range of applications including live cell imaging and cell membrane studies [1]. To perform CARS microscopy, the most common optical platform involves two electronically synchronised ultra-short pulsed lasers overlapped temporally and spatially. This complex system represents a significant financial outlay and the stability of the system is almost entirely dependent upon the electronic locking mechanism. However, for CARS microscopy to become routinely applied in life sciences research, simplified photonics technology is clearly required. As a consequence of this need, we demonstrate a CARS imaging system based on a synchronously pumped ultra-short pulsed optical parametric oscillator (OPO). This approach overcomes the need for two platform laser sources and electronic synchronization yet provides high power output and a wide wavelength tuning range to facilitate a wide range of CARS imaging experiments.

A Ti:Sapphire source (Coherent Mira Dual) operating at a 76MHz repetition rate served as the pump source for the OPO and provided a maximum average power at the PPLN crystal of ~1W. By virtue of the Ti:Sapphire design, ps- and fs-pulsed operation was possible, therefore allowing pumping of the OPO with ps- or fs-pulses. The multi-period crystal led to a signal and idler wavelength tuning range from ~0.94 μ m to ~1.19 μ m and ~2.01 μ m to ~3.61 μ m respectively. With a ps-pulsed pump, the maximum power at the signal mW with a pump power of 970mW at 747nm. The wavelength tuning characteristics of the OPO through varying the crystal temperature, crystal periods and cavity length will be presented, along with the performance of the fs-pulsed OPO. Considering the signal output of the OPO and a fraction of the Ti:Sapphire laser as the Stokes beam and the pump beam respectively, the 1800 to 4200 cm^{-1} of the Raman spectrum is accessible when used in conjunction with a modified Olympus FV300 laser scanning microscope system.

We report the application of the system for CARS imaging of mouse fibroblast cells to study aggregation and prevalence of lipids, with excitation ~2900 cm^{-1} . We will also give an overview of future work, which will involve correlations of the GFP-tagged receptor localisation with lipid raft structures in live polarized migratory cells to determine both lipid raft size and environment. Development of such an assay is vital to understanding the true role of lipid rafts in partitioning signalling events during cell migration.

[1]. C. L. Evans & X. Sunney Xie. Annual Rev. of Analytical Chem. 1 883, (2008).