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# Simple, High Performance Multi-modal Coherent Anti-Stokes Raman Scattering (CARS) Microscopy based on a Two-Photon Microscope

A. Pegoraro,[1,2] A. Ridsdale, [1] R. K. Lyn, [1,3] John P. Pezacki, [1,3] and A. Stolow[1,2]

1. Steacie Institute for Molecular Sciences, National Research Council. Ottawa, ON K1A 0R6
2. Department of Physics, Queen's University, Kingston, ON, K7L 3N6
3. Department of Chemistry, University of Ottawa, Ottawa, ON, K1N 6N5

Coherent anti-Stokes Raman scattering (CARS) microscopy [1] requires two input laser colours, called 'Pump' and 'Stokes' pulses; the frequency difference between them being tuned to the Raman resonance of interest. For live cell and tissue imaging, much of the focus has been on using picosecond lasers, either from two independent synchronized lasers or from an optical parametric oscillator (OPO) [2]. The use of femtosecond lasers has been explored, but more often in terms of multiplex CARS microscopy of cells [3, 4] and not tissue imaging. We have successfully employed a single femtosecond oscillator to perform tissue and live cell imaging with good spectral resolution, based upon conversion of a two-photon microscope.

Our laser source is a Ti:Sapphire oscillator centered at 800 nm with a pulse length of 60 fs, average power of 500 mW, and 80 MHz repetition rate (Tsunami, Spectra Physics). The microscope we use is a modified Olympus Fluoview 300 laser scanning confocal microscope. To generate our Stokes light, we use a two-zero-dispersion-point photonic crystal fiber (PCF), as depicted in figure 1. Although broad bandwidth, femtosecond pulses are coherent and therefore we can use optical pulse shaping to enhance the spectral resolution. Our first approach was to use a chirped Stokes pulse with a (unchirped) femtosecond Pump pulse. The latter 'gates' the Stokes pulses spectrum, giving us an effectively much smaller excitation bandwidth for the CARS process. This idea can be further extended by chirping both the Pump and Stokes pulse. If the chirp rate is identical for the two pulses, this allows us to put all pulse energy on resonance giving high spectral resolution. We then tune the Raman mode being probed by changing the time delay between the two pulses. This is illustrated in figure 2 below: we can clearly resolve the two peaks in methanol using chirped pulse CARS. The main advantage of this technique is that by tuning the chirp, we can trade-off spectral resolution for intensity in the CARS signal. For molecules in condensed phases with broad Raman line widths, this is a favorable situation. Furthermore, we can use shorter pulses to enhance signal from either two-photon fluorescence or second harmonic generation giving maximum flexibility for multimodal imaging. Finally, by increasing the chirp, the nonresonant signal will drop faster than the resonant signal, improving contrast for imaging. In Figure 3 we illustrate simultaneous two-photon fluorescence and CARS imaging of hepatitis C infected hepatocytes. In general, our results are favourably comparable to those obtained with two picosecond laser pulses.

In sum, we present a simple modification that can be made to any existing two-photon microscope, transforming it into a high performance multi-modal CARS microscope.

References

[1] A. Zumbusch, et al. *Phys. Rev. Lett.* 92 (1999) 4142.

[2] F. Ganikhanov, et al. *Opt. Lett.* 31 (2006) 1292.

[3] J-X Cheng, et al. *J. Phys. Chem. B* 106 (2002) 8493.

[4] K. P. Knutsen, et al. *J. Phys. Chem. B* 110 (2006) 5854.

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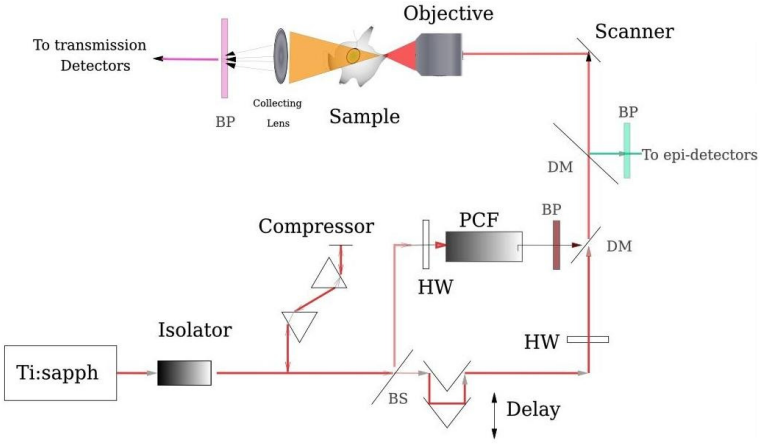


FIG. 1 Schematic of apparatus. A single femtosecond oscillator is used with the Stokes pulse being generated in a PCF. BS = beamsplitter, HW = half waveplate, BP = bandpass, DM = dichroic mirror.

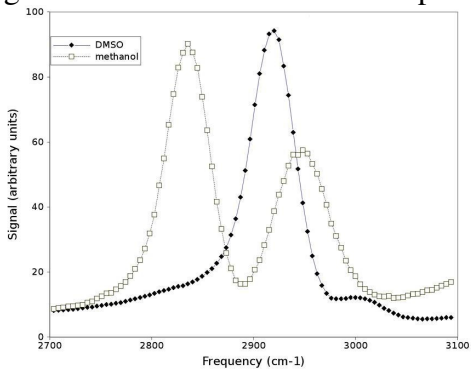


FIG. 2. Doubly-chirped CARS microscopy of methanol, and dimethyl-sulfoxide (DMSO) demonstrating that the doubly chirped technique offers enhancement of spectral resolution.

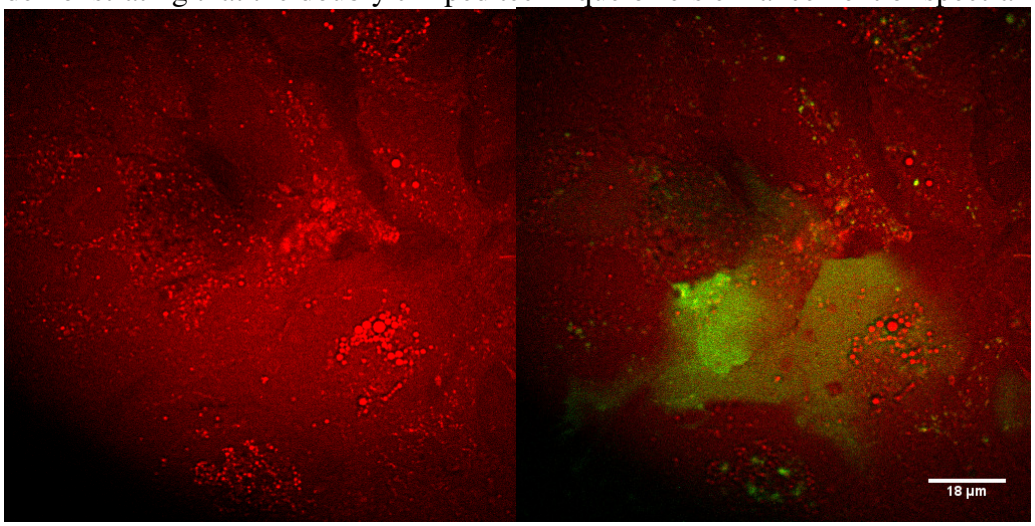


FIG. 3. Multi-modal CARS image of live hepatocytes. Red is forward collected CARS and green is epi-detected two photon fluorescence from GFP expressed by HCV infected cells.