Ultrabroadband coherent anti-Stokes Raman scattering microscopy

J.P.R. Day, M. Bonn, FOM Institute AMOLF, Amsterdam, The Netherlands, (November 2011)

Application Note

Introduction

Ultrabroadband coherent anti-Stokes Raman scattering (CARS) microscopy is a recently developed technique that generates quantitative, microscopic images of a sample based on vibrational contrast alone [1,2]. As such, it is a label-free, micro-spectroscopic technique that combines the benefits of spontaneous Raman microscopy (acquisition of full vibrational spectra) with imaging CARS microscopy (rapid acquisition of images). It has been applied in fields as diverse as cellular uptake and heterogeneous catalysis.

The CARS process can be compared to vibrational spontaneous Raman microscopy; indeed, both techniques probe the same Raman-active vibrational modes, as shown in Figure 1. However, CARS microscopy also offers some additional benefits over spontaneous Raman microscopy. The CARS signal is emitted at a higher frequency than the incident light fields (see Figure 1b), with the result that the CARS signal is not encumbered by a fluorescent background. As CARS is a stimulated process, the CARS signal is typically more intense than the spontaneous Raman signal, which enables rapid imaging. Lastly, CARS is also a nonlinear process based upon four-wave mixing, which engenders higher spatial resolution and inherent confocality without the need for a confocal pinhole.

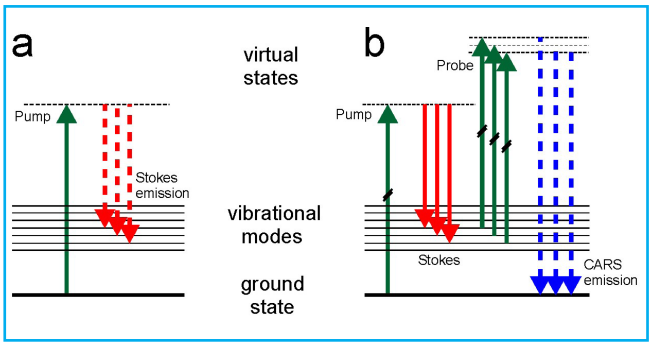

Figure 1: Energy level diagrams for a) spontaneous Raman spectroscopy and b) CARS spectroscopy. Solid lines represent incident light fields, and dotted lines represent emitted light fields.

The spectral bandwidth is determined by the bandwidth of the Stokes laser pulse. In the past, this bandwidth was restricted by the availability of suitable laser sources, but recent developments in supercontinuum generation in nonlinear fibers now allow the entire vibrational spectrum from the Rayleigh line up to 4000 cm^{-1} to be accessed.

Experimental Setup

The ultrabroadband CARS microscope employs a dual-output supercontinuum source (LEUKOS-CARS, Leukos Innovative Optical Systems) coupled to an inverted microscope (Eclipse Ti-U, Nikon) that is equipped with a high-speed, 3-axis piezo stage (Nano-PDQ 375 HS, Mad City Labs). The CARS photons are generated in the near IR (750-1064 nm) and thus the challenges for a detector are two-fold: the sensitivity of the CCD must be sufficiently high in this near-IR region and the readout speed of the CCD should be capable of spectrum rates in the kHz range. The Andor Newton DU920P-BR-DD employs a deep-depleted, back-illuminated CCD sensor in order to deliver high quantum efficiencies in the near IR without etaloning (Figure 2). The 3 MHz readout enables up to 1600 spectra to be acquired per second (Figure 3). The combination of this camera with an Andor Shamrock SR-303i-A-SIL spectrograph (600 lines/mm grating blazed at 1000 nm, protective silver coating of mirrors and grating for higher NIR reflectivity) allows rapid spectro-microscopic imaging with high signal-to noise ratios.

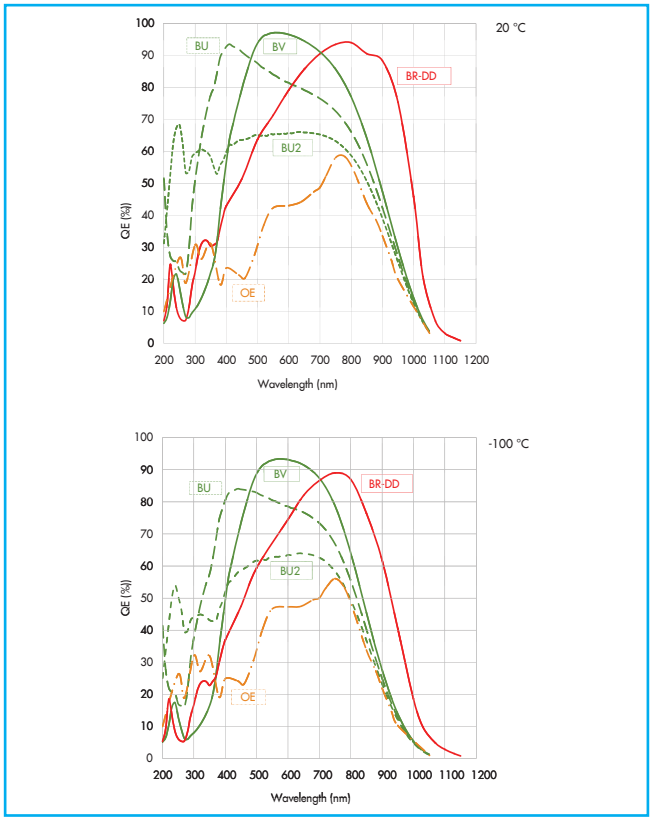

Figure 2: Quantum efficiency curves for various detector options.

Ultrabroadband coherent anti-Stokes Raman scattering microscopy

J.P.R. Day, M. Bonn, FOM Institute AMOLF, Amsterdam, The Netherlands, (November 2011)

Application Note

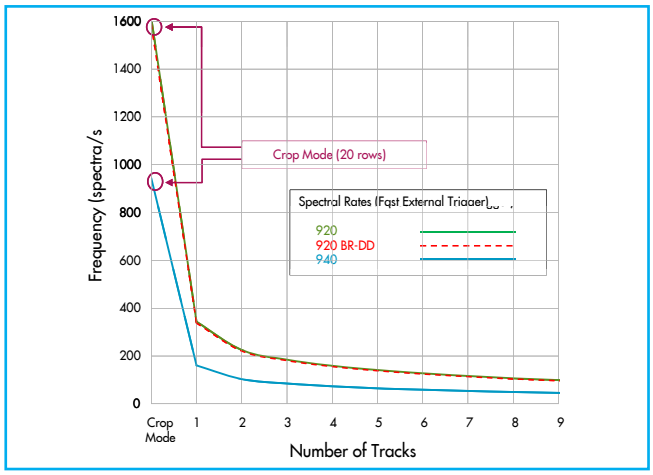

Figure 3: Readout rates for Andor Newton CCDs.

Results

A CARS spectrum from within a 3 µm polystyrene bead suspended in agar gel is shown in Figure 4. This spectrum was acquired in just 2 ms. These rapid acquisition speeds allow fast imaging: 2D reconstructions of the polystyrene beads are shown in Figure 5 based on seven different regions of the spectra (-3060 cm^{-1}, -2975 cm^{-1}, -2905 cm^{-1}, -2854 cm^{-1}, -1600 cm^{-1}, -1185 cm^{-1} and -1000 cm^{-1}). Crucially, all these images are acquired simultaneously in just 800 ms.

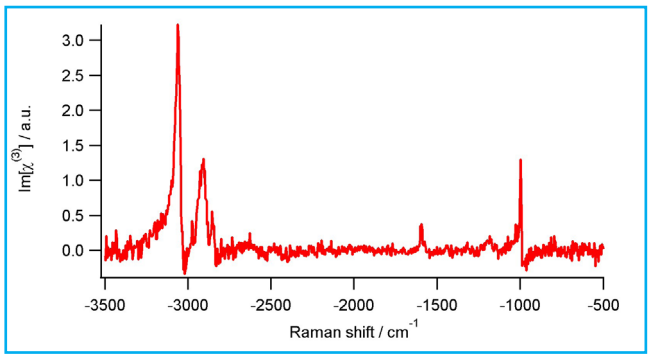

Figure 4: CARS spectrum of a 3 µm polystyrene bead. N.b. This spectrum has been subjected to phase retrieval by the maximum entropy method to convert the raw CARS spectrum into a quantitative Im[$\chi^{(3)}$] spectrum [2,3].

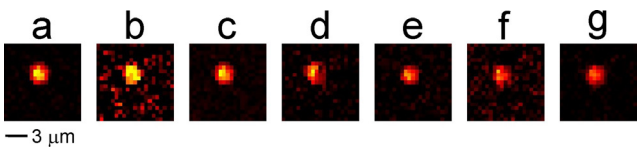

Figure 5: 2D reconstructions of a 3-µm polystyrene bead, obtained by integrating the a) -3060 cm^{-1}, b) -2975 cm^{-1}, c) -2905 cm^{-1}, d) -2854 cm^{-1}, e) -1600 cm^{-1}, f) -1185 cm^{-1} and g) -1000 cm^{-1} bands.

This simple polystyrene bead suspension serves to demonstrate the capabilities of ultrabroadband CARS, but the enhanced bandwidth becomes particularly useful for more complex samples, such as biological tissues and cells. In these samples, the ability to acquire full vibrational spectra rapidly throughout the image is invaluable for reliable assignment and assessment of the sample behaviour.

Outlook

The availability of CCD detectors with high NIR quantum efficiencies and high spectral rates, together with turn-key supercontinuum laser sources holds great promise in the development of ultrabroadband CARS microscopy for biological, chemical and material science studies. In particular, these advances enable the movement of CARS microscopes out of the dedicated laser laboratory to become a routine analysis tool.

References

[1] Quantitative CARS molecular fingerprinting of single living cells with the use of the Maximum Entropy Method. M. Okuno et al., Angewandte Chemie Int. Ed., 2010, 49, 6773-6777

[2] Quantitative coherent anti-Stokes Raman scattering, J. P. R. Day et al., J. Phys. Chem. B, 2011, 115, 7713-7725.

[3] Direct extraction of Raman line-shapes from congested CARS spectra, E. M. Vartiainen et al., Optics Express, 2006, 14, 3622-3630.

Contact

Prof. Dr. Mischa Bonn
Max Planck Institute for Polymer Research
Department of Molecular Spectroscopy
Ackermannweg 10
55128 Mainz
Germany

Phone: +49 (0)6131 379160
E-mail: bonn@mpip-mainz.mpg.de
Web: www.mpip-mainz.mpg.de