Wide-field broadband CARS microscopy

Chiara Ceconello[1], *Federico* Vernuccio[1], *Alejandro* De la Cadena[1], *Arianna* Bresci[1], *Francesco* Manetti[1], *Subir* Das[1], *Renzo* Vanna[2], *Giulio* Cerullo[1,2], and *Dario* Polli[1,2,*]

[1]Department of Physics, Politecnico di Milano, P.zza Leonardo da Vinci 32, 20133 Milan, Italy
[2]CNR Institute for photonics and nanotechnologies (IFN), P.zza Leonardo da Vinci 32, 20133 Milan, Italy

Abstract. Coherent anti-Stokes Raman scattering is an extremely powerful non-linear optical (NLO) microscopy technique for label-free vibrational imaging allowing for a detailed study of biological samples in their native state. To overcome the long acquisition times associated with raster sample scanning required in NLO microscopy, which impair real-time investigation of fast biological dynamics, we employ here wide-field signal generation over a large field of view, covering tens of micrometers. To this aim, we exploit an innovative approach based on the use of an amplified femtosecond ytterbium laser source delivering high energy (≈μJ) pulses in the near infrared. This enables the generation of stable broadband Stokes pulses to measure the entire fingerprint region of the molecular vibrational spectrum, the richest in chemical information. Our results pave the way for future translational applications and clinical diagnostics with video-rate imaging capabilities.

1 Introduction

Spontaneous Raman (SR) represents an extremely powerful investigation tool for life sciences allowing to characterize and map in 3D each component of a sample (like cells and tissues) in a label-free, remote and non-destructive manner. However, it suffers from a very low cross section, resulting in long acquisition times. The solution comes with coherent Raman scattering techniques that employ ultrashort pulses to promptly trigger the vibrational response [1]. Coherent anti-Stokes Raman Scattering (CARS) employs two narrowband sinchronized picosecond pulses (pump and Stokes) to probe a single vibrational mode by detecting the signal at the blue-shifted anti-Stokes frequency. Broadband (B)-CARS increases the amount of extracted information by employing a narrowband pump pulse and a broadband Stokes that allows to probe an entire vibrational spectrum, thus combining high acquisition speed of CARS with the chemical specificity of SR. Traditionally B-CARS microscopy is performed in raster scanning fashion: tightly focused laser beams generate signal in one pixel at a time which is then acquired in a snake-like motion with a spectrometer. It can take up to minutes to generate a single image, even at the state of the art 3ms pixel dwell time [2], thus preventing real-time investigation of fast biological dynamics. Wide Field (WF) illumination on the other hand, allows signal generation over a large field of view, which is then acquired single shot with the help of a sCMOS camera. Non phase-matching WF illumination [3] represents a simple configuration for effective generation of WF-CARS. In this work, we present a novel configuration of WF-CARS microscopy with broadband and video-rate imaging capabilites. We employ an amplified ytterbium laser at 1035-nm central wavelength and 2-MHz repetition rate that enables white-light continuum (WLC) generation in bulk media: a compact, robust, simple and alignment-insensitive technique, that presents excellent long-term stability and low pulse-to-pulse fluctuations [4]. Unlike standard CARS setups, the red-shifted source (1035 nm pump, 1050-1300 nm Stokes) reduce multi-photon sample photo-damage, allowing the use of higher laser intensities on the sample. Furthermore, the reduced repetition rate allows for higher pulse energies, thus higher peak intensities that generate a stronger B-CARS signal thanks to the non-linear nature of the optical effect. As a test sample we used 8 μm Polystyrene (PS) beads in a solution of Dimethyl sulfoxide (DMSO). The spectral nature of the signal was assessed by performing single wavelenght WF-CARS imaging. Video-rate WF-CARS was demonstrated by collecting low acquisition time images at 2ms integration time.

2 Materials and methods

As shown in Figure 1, an amplified Ytterbium laser source delivers ≈ 270 femtosecond pulses at 1035 nm central wavelength, 2-MHz repetition rate and ≈ 5W average power. A polarizing beam splitter separates the fundamental in two beams. The first one goes through an etalon to generate narrowband pump pulses with ≈ 1.1 nm bandwith (10 cm^{-1}) guaranteeing sufficient spectral resolution for the vibrational Lorentian peaks. The second provides high enough pulse energy (≈ 1.2 μJ) to efficiently generate a stable white light supercontinuum in

*e-mail: dario.polli@polimi.it

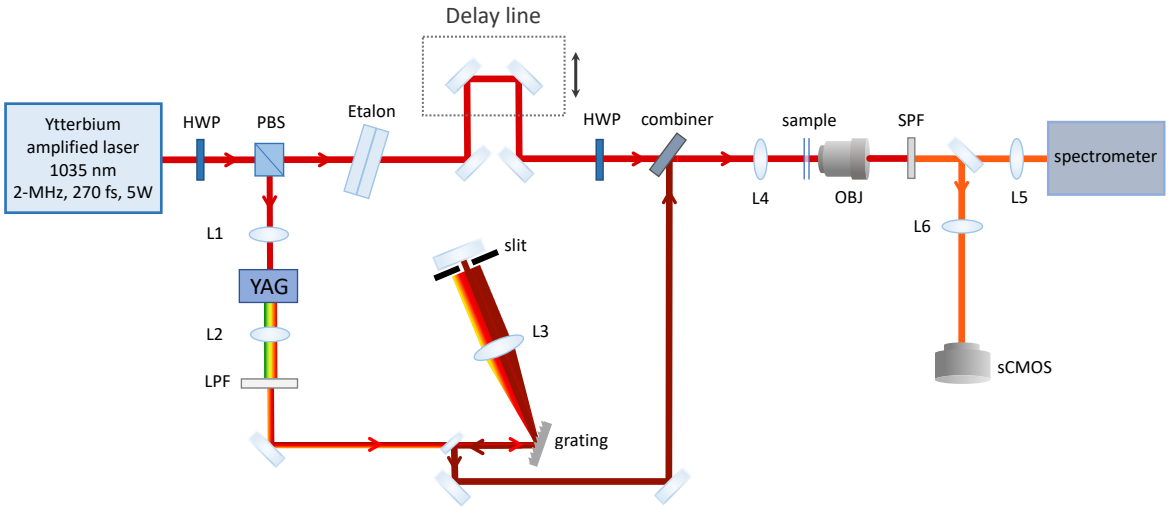

Figure 1. Scheme of the WF-CARS experimental setup. HWP: half-wave plate. PBS: Polarizing Beam Splitter. L1,L2,L3,L4,L5,L6: lenses. LPF: long-pass filter. OBJ: collection objective. SPF: short pass filter.

a 6mm thick YAG crystal. The fundamental is removed with a long pass filter that selects the red-shifted portion (1050-1300 nm) thus obtaining a broadband Stokes pulse that covers the whole fingerprint region (400-1800 cm $^{-1}$). In the Stokes path, a pulse shaper in 4f configuration with an adjustable mechanical slit mounted on a traslational stage allows for single-color selection. The two beams are then spatially and temporally recombined and loosely focused with a 100mm lens. The pump has a FWHM spot of 50-µm on the sample, with ≈210 mW average power, while the Stokes has a 60-µm spot and ≈10 mW average power. In this way it is possible to generate WF CARS signals from a 40-µm FWHM area, in a non-phase matched illumination configuration.

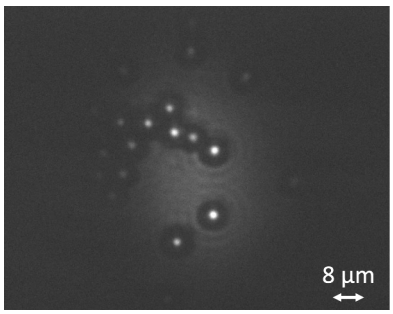

Figure 2. Wide-Field CARS image of 8-µm Polystyrene (PS) beads in a solution of Dimethyl sulfoxide (DMSO). 460x540 pixels, 2ms integration time.

The WF-CARS signal is collected with a 20x objective (NA=0.3), recorded with a 4.2 megapixels sCMOS placed after a short-pass filter to cut the Stokes and pump beams. WF-CARS imaging is performed single shot, acquiring the whole field of view at once. For our experiment we imaged 8-µm Polystyrene (PS) beads in a solution of Dimethyl sulfoxide (DMSO) at various integration times. The video-rate capabilities of the setup were assessed by performing imaging with the mechanical slit of the 4f pulse shaper kept open, thus generating WF-CARS signal in the Ω 647-976 cm^{-1} spectral range. 460x540 pixels images of PS beads in DMSO were acquired with 2ms integration time (Figure 2). We then proved the spectral nature of the signal by performing hyperspectral WF-CARS measurements. We obtained quasi single wavelength Stokes beam by accurately closing the mechanical slit in the 4f pulse shaper varying its color by shifting the slit position thanks to a micrometric traslational stage. By acquiring different frames at the different Stokes wavelengths, we built three-dimensional hyperspectral WF-CARS data-cubes as a function of the vibrational frequency for the entire field of view. These results confirm the capability of generating stable WF-CARS signal, with forthcoming video-rate and broadband imaging possibilities.

References

[1] Zhang C., Cheng J.X., Perspective: Coherent Raman scattering microscopy, the future is bright, APL Photonics **3**, (2018)

[2] Camp C.H.J., Lee Y.J., Heddleston J.M., Hartshorn C.M., Hight Walker A.R., Rich J.N., Lathia J.D., Cicerone M.T., High-speed coherent Raman fingerprint imaging of biological tissues, Nature Photonics **8**, (2014)

[3] Lei M., Winterhalder M., Selm R., Zumbusch A., Video-rate wide-field coherent anti-Stokes Raman scattering microscopy with collinear nonphase-matching illumination, Journal of Biomedical Optics **16**, (2011)

[4] Dubietis, A., Tamošauskas, G., Šuminas, R., Jukna, V. Couairon, A., Ultrafast supercontinuum generation in bulk condensed media, Lith. J. Phys. **57**, (2017)