Broadband Fourier-Transform Stimulated Raman Scattering Microscopy

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Coherent Raman Scattering (CRS) microscopy is capable of non-invasive, label-free imaging of tissues and cells, based on their intrinsic vibrational response. Coherent Anti-Stokes Raman Scattering (CARS) and Stimulated Raman Scattering (SRS) are the two most commonly employed CRS techniques. With respect to CARS, SRS presents several advantages: the signal is proportional to species concentration and is free of non-resonant four-wave-mixing. However, SRS is technically very challenging, as it requires high-speed modulation and synchronous lock-in detection to measure the tiny (10^{-4} or less) differential transmission ($\Delta T/T$) signal sitting on top of a large linear background. Broadband SRS microscopy is very challenging and, typically, multiplex SRS is performed with narrowband pulses, by rapidly scanning the pump-Stokes frequency detuning.

Here we demonstrate a new approach to broadband SRS based on time-domain Fourier transform (FT) spectroscopy. Our approach blends the very high sensitivity of single-channel lock-in balanced detection with the spectral resolution of FT spectroscopy. After the sample, the transmitted Stokes is sent to a home-made Fourier transform spectrometer based on a passive birefringent delay line [1], allowing us to create two replicas of the Stokes pulse, with perpendicular polarizations, whose delay can be finely controlled with exceptional (<λ/300) path-length stability and reproducibility. A Wollaston prism and a balanced photodiode are then used to record two out-of-phase interferograms. The output of the detector, measured with an analog-to-digital converter (ADC) as a function of the replicas delay, is the Stokes pulse interferogram I_{STOKES} (Fig. 1(a)), whose FT (shown in panel (c) as a function of pump-Stokes frequency detuning) gives the Stokes spectrum. Panel (b) shows an interferogram of the pump-induced Stokes intensity variation ΔI_{STOKES}, extracted by the single-channel lock-in amplifier, for an isopropanol solution. The signal extends over much longer delays, due to the interference of the Stokes pulse with the field radiated by the nonlinear polarization generated in the medium, which dephases on the picosecond timescale. The FT of ΔI_{STOKES}, displayed in panel (d), shows the three characteristic peaks of isopropanol at ~2870, ~2930 and ~3000 cm^{-1}.

We applied our transient FT spectrometer also to SRS microscopy. We imaged a mixture of polymethyl methacrylate (PMMA) and polystyrene (PS) spherical beads with 6-μm and 3-μm diameter, respectively [2]. The measured three-dimensional dataset as a function of sample position and Raman shift can be analyzed using a Multivariate Curve Resolution-Alternating Least Square algorithm, that decomposes the experimental dataset in a set of spectra and concentration vectors. In this way, we correctly identified two principal components only, with spectra perfectly matching those of PMMA and PS. Figure 5(e) reports the corresponding concentration of the two components and provides a clear chemically selective map of the sample. Our novel approach paves the way to high speed chemical identification of biomolecules via their broadband coherent Raman response.

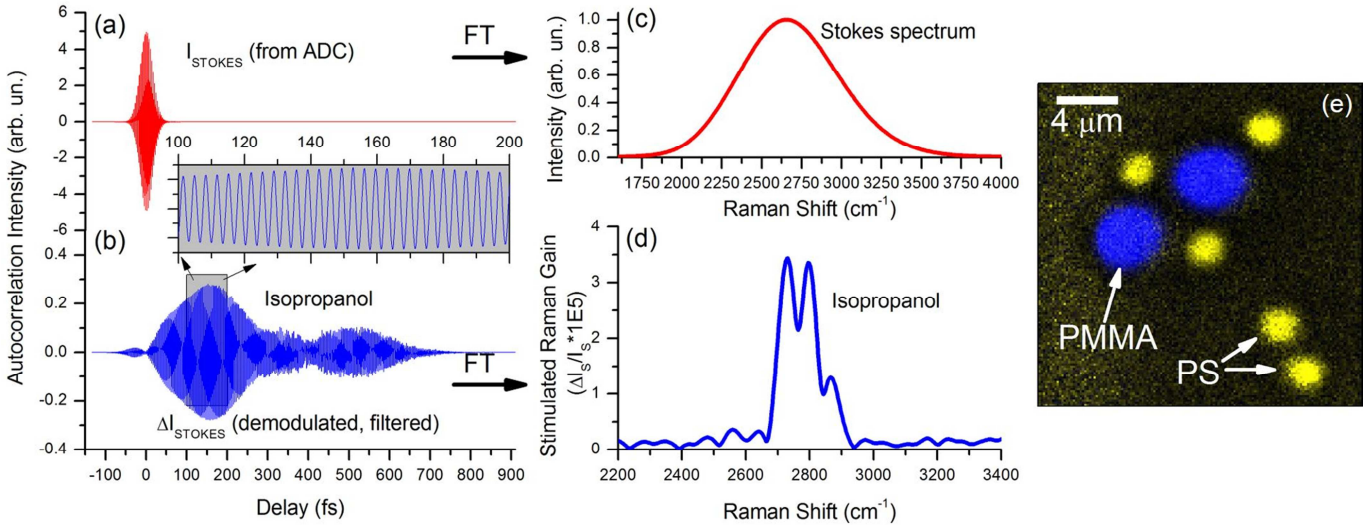

Fig. 1. (a) interferogram of the Stokes pulse measured by scanning TWINS; (b) interferogram of the pump-induced Stokes intensity variation in isopropanol, demodulated by the lock-in; (c) FT of (a), yielding the spectrum of the Stokes pulse; (d) stimulated Raman gain spectrum of isopropanol, obtained by normalizing the FT of (b) by the FT of (a).(e) FT-SRS imaging of polymer beads.

References

[1] D. Brida, C. Manzoni, and G. Cerullo, Opt. Lett. **37**, 3027 (2012).

[2] J. Réhault, F. Crisafi, V. Kumar, G. Ciardi, M. Marangoni, G. Cerullo and D. Polli, Opt. Expr. **23**, 25235 (2015).