Advances in Coherent Raman Scattering Microscopy

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Coherent Raman Scattering (CRS) microscopy is gaining a growing recognition in biomedical optics, thanks to its capability of non-invasive, label-free imaging of tissues and cells. CRS is a class of third-order nonlinear optical techniques using two synchronized laser pulses at frequencies ω_p (pump) and ω_S (Stokes). When the difference between pump and Stokes frequencies matches a vibrational frequency Ω of a molecule, i.e., $\omega_p - \omega_S = \Omega$, all the molecules in the focal volume vibrate in phase, creating a vibrational coherence which enhances the Raman response by many orders of magnitude with respect to spontaneous Raman. CRS has the following advantages: (i) the signal is generated only in the focal volume, allowing three-dimensional imaging; (ii) working out of electronic resonance, it minimizes photo-damage to biological samples; (iii) compared to fluorescence microscopy, it works on the pristine tissue, avoiding sample labelling which often prevent application in a intraoperative context.

The two most widely employed CRS techniques are Coherent Antistokes Raman Scattering (CARS) [1] and Stimulated Raman Scattering (SRS) [2]. In CARS the vibrational coherence is read by a further interaction with the pump beam, generating a coherent radiation at the anti-Stokes frequency $\omega_{aS} = \omega_p + \Omega$. In SRS the coherent interaction with the sample induces stimulated emission from a virtual state of the sample to the investigated vibrational state, resulting in Stokes-field amplification (called Stimulated Raman Gain, SRG) and simultaneous pump-field attenuation (called Stimulated Raman Loss, SRL). CARS has the advantage of being virtually background-free, since the emitted signal has a frequency ω_{aS} differing from those of pump and Stokes. On the other hand, it suffers from the so-called non-resonant background (NRB) generated both by the molecular species under study and by the surrounding medium, according to a four-wave mixing scheme. The NRB does not carry any chemically specific information and, when the concentrations of the target molecules are low, distorts and even overwhelms the resonant signal of interest. In addition, CARS signal scales as N^2, where N is the number of oscillators in the focal volume, so that its sensitivity rapidly drops with decreasing oscillator concentration, making it difficult to detect the less abundant biomolecules. The SRS signal is proportional to the imaginary part of the third order susceptibility and is thus inherently free from NRB, which is a real quantity. Furthermore, SRS scales linearly with N, allowing the detection of weakly concentrated species. On the other hand, SRS requires the detection of a weak differential transmission signal (SRG or SRL) sitting on the large (and noisy) background given by the Stokes (or pump) light. Extraction of this signal requires the use of sophisticated techniques, involving high-speed modulation and lock-in detection, which are challenging to implement at high speeds.

For a widespread adoption of CRS technique in the biomedical community, a few problems remain to be solved: (i) laser sources used to generate pump and Stokes pulses are typically complex and expensive; (ii) it is hard to achieve shot-noise-limited sensitivity in SRS; (iii) CARS and SRS typically work at a single frequency, thus providing a limited chemical sensitivity which is not sufficient to distinguish the different components within complex heterogeneous systems, such as cells and tissues, with spectrally overlapping chemical species. This talk will review our recent work on CRS techniques, aimed at overcoming these hurdles. We have developed several simplified laser systems generating the synchronized pump and Stokes pulses, including compact, turn-key fiber-format lasers [3, 4] and a synchronization-free system based on nonlinear frequency conversion [5]. We have introduced a novel CRS technique, called balanced-detection Raman-induced Kerr effect (BD-RIKE) [6], which employs a balanced-detection architecture to sensitively measure the polarization rotation of the Stokes/pump field. Balanced detection allows for an intrinsic rejection of laser noise and provides self-heterodyne amplification of the nonlinear Raman signal. Finally, we have demonstrated a new method for broadband SRS microscopy based on time-domain Fourier transform (FT) detection of the SRG spectrum [7]. This approach blends the very high sensitivity of single-channel lock-in balanced detection with the spectral coverage and resolution afforded by FT spectroscopy.

References

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