Technology note

HÜBNER Photonics

SERDS to Silence Fluorescence

Revealing Hidden Raman Signals with New Dual-line Laser

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Raman spectroscopy is a powerful analytical technique valued for its non-invasive sampling, high level of molecular specificity and spatial resolution, making it ideal for applications such as pharmceutical and semiconductor process monitoring, material science, and biomedical research. However, its utility is often hindered by the presence of strong fluorescence emission from the sample, which hides the inherently much weaker Raman scattering signal. Shifted Excitation Raman Difference Spectroscopy (SERDS) can offer a solution to this problem by effectively eliminating the background fluorescence. The technique uses two narrow-linewidth lasers with slightly different emission wavelengths. As the Raman peaks spectrally follow the excitation while fluorescence remains largely unchanged, subtracting the two spectra effectively removes the fluorescence background, revealing the underlying Raman signal. This article outlines the principles and practical benefits of SERDS and presents a permanently aligned, compact and robust dual-line laser solution at 785 nm for simplifying SERDS related acquisition.

Principles of SERDS

Raman spectroscopy studies the vibrational and rotational modes (and other low frequency ones) of a sample based on light scattering, a process in which photons promote the system into a virtual state rather than an excited electronic state (e.g. S1 in Figure 1). As the system relaxes, it can scatter photons with less energy (Stokes), equal energy (Rayleigh), or more energy (Anti-Stokes) than the incident light, depending on the vibrational or rotational state involved in the transition.

The probability of Raman scattering is inherently low and follows an inverse fourth-power dependence on the excitation wavelength ($\propto 1/\lambda^4$). This means shorter wavelengths (higher frequencies) result in stronger Raman signals, prompting many spectroscopists to use green lasers of 532 nm.

However, shorter wavelengths often overlap with electronic transitions in the sample, leading to fluorescence which can overshadow the weak Raman signal. To mitigate this, longer excitation wavelengths such as 785 nm are commonly used. While this produces a weaker Raman signal due to the $1/\lambda^4$ dependence, it also reduces the influence of fluorescence. To compensate for the reduced signal strength, plasmonic enhancement techniques like Surface-Enhanced Raman Spectroscopy (SERS) and Tip-Enhanced Raman Spectroscopy (TERS) are often employed.

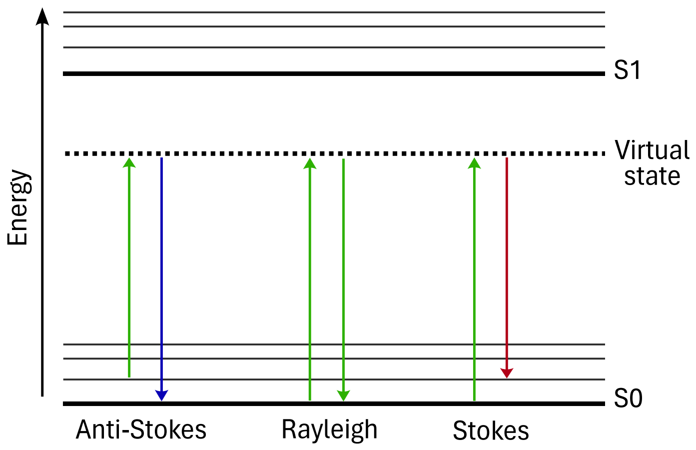

Figure 1. Vibrational transitions in Raman spectroscopy. Rayleigh scattering corresponds to the elastic scattering of the incoming laser light. Stokes and anti-Stokes are inelastic scattering processes where energy of the incident light is respectively lost to or gained by vib-rot transitions.

Another strategy is to isolate the Raman signal in fluorescence-dominated spectra by Shifted Excitation Raman Difference Spectroscopy (SERDS) or also referred to as Differential Raman Spectroscopy (Figure 2). This method produces a Raman spectrum using two laser excitations with slightly different wavelengths (typically 0.5–1 nm apart). Raman peaks shift according to the excitation wavelength, while the interfering fluorescence will remain constant at small shifts. Subtracting the spectra thus cancels the fluorescence and systematic noise (e.g. stray light), producing a difference spectrum that can be reconstructed into a clean Raman spectrum.

Additional post-processing, such as baseline correction or spectral fitting, may however be required, especially in cases of photobleaching or laser instability. These phenomena could namely cause the fluorescence background to alter between the two shifted excitation spectra and consequently introduce artifacts in the difference spectrum. Despite such challenges, SERDS remains a powerful tool for extracting Raman signals from strongly fluorescent samples.

Most current SERDS experimental setups are custom-built and rely either on complex tunable lasers for wavelength shifting or on two separate single-frequency lasers, where both approaches tend to be costly and bulky. These laser sources must meet several stringent criteria: they require narrow linewidths for clear spectral separation for SERDS as well as high spectral resolution, and they must operate in true single longitudinal mode (SLM) without mode-hopping. Any instability in wavelength or power can introduce noise that the SERDS subtraction cannot eliminate, leading to significant artifacts in the reconstructed Raman spectrum. As SERDS performance hinges on the precision and stability of the excitation source, careful selection of the laser system is therefore crucial.

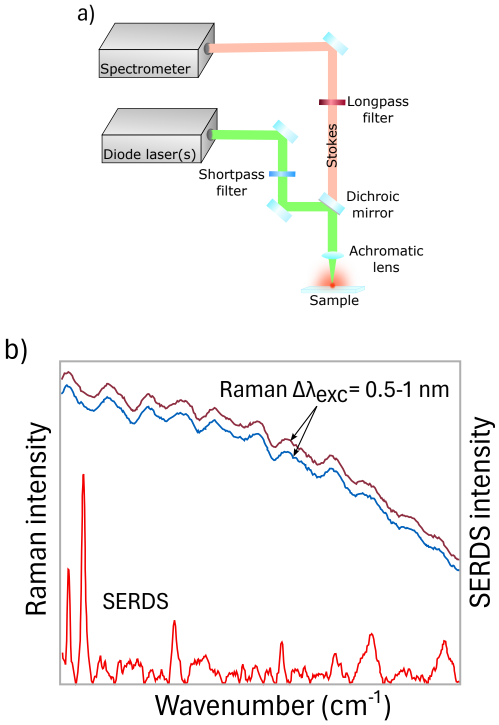

Figure 2. Schematic representation of SERDS. a) setup where a tuneable laser is used as excitation source. The excitation occurs with one frequency at a time and reaches the sample through a short-pass filter. The scattered light (here Stokes) is detected by a spectrometer after passing through a dichroic mirror and a long-pass filter. b) SERDS signal obtained from two shifted excitation (Δλexc) Raman signals.

New laser technology for simple SERDS

For SERDS to become widely applied to fluorescent heavy samples, the quality of the results must be worth the time spent measuring and conducting the sometimes several steps of data treatment. As previously implied, the results will depend on the use of two spectrally separated narrow-linewidth lasers, free from mode-hopping and spectral drift. The narrow-linewidth and small spectral drift (both preferably <1 pm) are important to not impair the spectral resolution of the results and for SERDS. In addition, the two sources need to have a relatively small (0.5.-1 nm) but very stable shift in wavelength. Moreover, a high level of power stability and single-transversal mode beam quality are needed for short acquisition times, high spatial resolution and optimum signal-to-noise ratio and subsequent fluorescence cancellation. All these laser requirements can now be conveniently accessed thanks to new development of the Cobolt Skyra^{TM} multi-line laser platform.

Figure 3. Compact laser housings of Cobolt's Skyra^{TM}, an all-integrated 1-box solution for two narrow-linewidth lasers at 785 nm with a fixed 0.5 nm spectral shift. With free beam output (left) or with fiber-coupled output (right).

The Cobolt Skyra^{TM} is a compact, permanently aligned multi-line laser system, now featuring two integrated narrow-linewidth, frequency-stabilized lasers. Centered near 785 nm with a 0.5 nm spectral shift, each laser delivers 100 mW of power (Figure 4). Both lines has a FWHM linewidth under 0.6 pm, achieved via stable frequency-locking alignment and precise cavity temperature control.

This new Cobolt Skyra™ dual-line 785 nm offers exceptional performance stability, with <3% power fluctuation over 8 hours, <0.3% RMS noise between 250 Hz - 2 MHz and a wavelength stability of 0.15 pm/°C (20-40 °C) and all this from an all-integrated single-box laser system of the dimensions 134 x 70 x 48 mm. This performance stability is enabled by the proprietary HTCure™ process, for robust and high precision assembly of miniaturized optics on thermo-mechanically stabilized platforms. With its compact size and high level of spectral precision, the Cobolt Skyra™ dual-line 785 nm laser makes the application of SERDS technology simpler and more accessible than previously, and even opens the door to portable, in-field Raman analysis of demanding highly fluorescent materials.

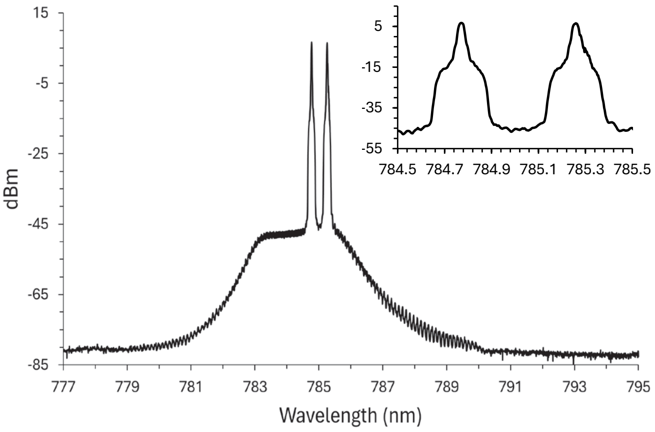

Figure 4. Optical spectra of a Cobolt Skyra™ dual-line 785 nm, optimized for SERDS. (Note: The spectral linewidth is limited by the resolution of the spectrum analyser and therefore not to scale.)

Conclusions

The introduction of the Cobolt Skyra™ dual-line 785 nm can enable development of compact, robust and cost-effective SERDS solutions for revealing Raman spectra in fluorescence-heavy environments. Its stability, narrow-linewidth, and dual-wavelength capability support efficient fluorescence cancellation and accurate signal reconstruction—paving the way for wider SERDS adoption.

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About HÜBNER Photonics

HÜBNER Photonics offers the full range of high performance single and multiline Cobolt lasers, the CW tunable laser C-WAVE, VALO femtosecond lasers, Ampheia fiber amplifiers along with a full selection of C-FLEX laser combiners. HÜBNER Photonics has become a preferred supplier of lasers to major instrument manufacturers and leading research labs for cutting-edge applications in the areas of fluorescence microscopy, flow cytometry, Raman spectroscopy, metrology, holography, nanophotonic and quantum research.