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Fluorescence suppression in resonance Raman spectroscopy using a high-performance picosecond Kerr gate

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A high-performance Kerr gate designed for the suppression of fluorescence in both time-resolved and steady-state resonance Raman spectroscopy is described and its performance illustrated. This is an improved version of a system described recently, with superior extinction ratio, higher throughput and wider usable spectral range. Specially designed polarizers are an essential feature of the system. The gate opens for ~4 ps at 1 kHz repetition rate, throughput in the open state is up to ~40% (excluding Fresnel losses on optical elements), the extinction ratio in the closed state is 10^{-5} and the usable spectral range is 300–700 nm with a single set of polarizers. The Kerr gate is driven by ~500 μJ, 1 kHz, 1 ps laser pulses at 800 nm and we believe that it is currently the most powerful device in operation for the temporal rejection of fluorescence from Raman spectra. It increases the general applicability of Raman spectroscopy as illustrated by picosecond time-resolved resonance Raman spectra of the intramolecular charge transfer state of 4-dimethylaminobenzonitrile and by the ground-state Raman spectrum of unleaded petrol at 400 nm probe wavelength.

INTRODUCTION

Raman spectra obtained using a probe beam wavelength matched to a molecular absorption band have exceptional sensitivity and selectivity. The resonance greatly enhances the Raman signal from individual transition moments of the specific molecule to be studied. Unfortunately, fluorescence is often also strong, limiting observations in ground-state studies to particularly strong Raman bands. In many cases the probe beam [and the pump beam in time-resolved resonance Raman (TR^3) experiments] may induce fluorescence in contaminants or the solvent, and in biological or industrial cases particularly the concentration of the molecule under study may be low. The normal method to deal with these problems is to make background subtractions (solvent only, or probe-before-pump), but Raman signals are typically very weak and easily swamped by noise induced by fluorescence, which may be 10^6–10^8 times stronger than the signal.[1] Techniques proposed to deal with this problem include shifted excitation Raman difference spectroscopy[2] (SERDS), polarization modulation,[3] shifted spectra,[1,4] Fourier transform filtering[1] and temporal gating.[5–9] The temporal gating technique uses the main distinguishing feature of Raman scattering, its promptness, and probably has the most promise. A short pulsed Raman signal is generated using a pulsed laser and the longer lived fluorescence is rejected in the time domain. Simultaneous recording of the signal over a range of wavelengths is usually employed. Very short gating times were achieved by Tahara and Hamaguchi,[6] who used a streak camera with effective time gating resolution of ~10 ps limited by trigger jitter. A similar concept but relying on an optical Kerr gate[10–14] was proposed and demonstrated in ground-state Raman spectroscopy by Deffontaine *et al*.[15] using 25 ps gating pulses. This latter concept holds certain advantages over the streak camera owing to simplicity of the design and inherent absence of jitter problems allowing potential access to shorter gating times.

We have recently demonstrated the feasibility of Kerr gate fluorescence suppression with ~3 ps time resolution in Raman spectroscopy.[16] The prototype device had a throughput of about 30% (excluding Fresnel losses on optical elements) and extinction ratio 10^{-4} in the usable spectral range of 630–700 nm. However, we recognized the need and scope for improvement in order to detect signals from much

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weaker Raman scattering systems and to increase its spectral range greatly in order to use resonance enhancement for a wide range of molecules. Raman signals are inherently very weak: in our conventional ps-TR^3 system[17] many molecules of chemical interest yield only one detected Raman photon per 100–1000 laser pulses, per Raman band. Consequently, not only are the extinction ratio and opening time of the gate important but also the efficiency of transmission through the gate must be high in order to keep acquisition times to an absolute minimum. Here we present our improved design and characterize the performance of the enhanced device. The specifications are transmittance in the open state of ~40% (excluding Fresnel losses on optical elements), a higher rejection ratio of 10^{-5} and a substantially wider usable spectral range of 300–700 nm attained with a single pair of polarizers. This is achieved at the expense of a marginally lower temporal resolution, ~4 ps.

DESCRIPTION

The basic principle of the optical Kerr gate was described in a previous paper.[16] The optical arrangement of the improved system is shown in Fig. 1(a). Briefly, the Kerr gate consists of two crossed polarizers and a Kerr medium. In the closed state, light collected from the sample and collimated in an optical train is effectively blocked by the crossed polarizers. Coincident in time with the Raman scattered light from the sample, a short gating pulse of 800 nm wavelength that bypasses the polarizers creates a transient anisotropy within the Kerr medium by the optical Kerr effect. The gating beam is polarized at 45°. The intensity is adjusted to create an effective $\lambda/2$ waveplate and rotate the polarization of the light from the sample by 90°, allowing it to be fully transmitted through the cross polarizer for the duration of the gating pulse.

The improved Raman collection system consists of a parabolic collection mirror ($f^{\#} = 0.8$) of 50 mm diameter, image relay fused-silica lenses, a Kerr cell and two calcite

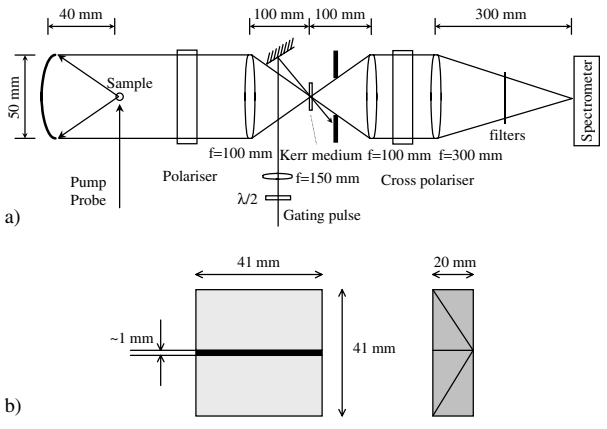

Figure 1. (a) Optical arrangement of the Raman collection system and the optical Kerr gate. The angle between the gating pulse and optical axis is ~10°. (b) Schematic diagram of the first segmented polarizer.

Glan Taylor segmented polarizers. The 800 nm gating pulse is weakly focused into the Kerr cell to a diameter of ~1–2 mm using a 150 mm focal length lens, with the beam waist placed in front of the cell to keep light intensity below the threshold for white light continuum generation and to reduce self-focusing and stimulated Raman effects. The Kerr cell containing static CS_2 was custom made with a 2 mm pathlength and ultra-thin (300 μm) Spectrosil B walls, to reduce self-focusing effects within the walls. The polarizers were of 41 × 41 mm aperture, manufactured to our specifications by Halbo Optics, replacing earlier sheet polarizers. The first polarizer [detailed in Fig. 1(b)] consists of two 20 × 41 mm segments of 20 mm thickness. This choice, rather than a single unit of twice the thickness, keeps group velocity dispersion well within the response time of the gate over the spectral range used in a typical Raman experiment (~1000 cm^{-1}). The choice of a 41 × 41 mm aperture is a compromise between the cost and optimum Raman throughput, with ~14% loss due to clipping on the polarizer being tolerated. The optical thickness of the polariser is uniform to within ±50 μm to keep the transit time variation of the Raman signal within the gate response time. The other parameters are extinction coefficient 10^{-5}, flatness $\lambda/8$, angular deviation of the transmitted beam <3 min of arc, peak transmission >88% and useful acceptance field angle >7°. The internally reflected beam is blocked on interfaces between the polarizer segments. The second polarizer placed after the Kerr cell is manufactured to the same specifications except that it has relaxed thickness uniformity to within ±1 mm as the temporal stretching of the Raman flux is not relevant in this case. For economic reasons this polarizer consists of four 20 × 20 mm segments instead of two longer ones. For ease of alignment and optimization the polarizers are mounted on rotational stages controlled by micropositioners.

The $f = 10$ cm lens placed immediately after the first polarizer images the sample interaction region into the Kerr cell. The rays emerging from the Kerr medium are then re-collimated by the second $f = 10$ cm lens, passed through the cross polarizer and imaged on to spectrometer entrance slit using a 30 cm focal length lens with the f-number ($f^{\#} = 6$) matching that of the three-stage spectrometer (Triplemate). To optimize the coupling of the horizontally oriented sample interaction zone to the vertical spectrometer slit, the scattered light was flipped by 90° using a pair of flat mirrors placed after the second polarizer. A laboratory-built Triplemate filter stage by-pass option was used in the experiments described here to optimize transmission. The probe laser line was blocked using a holographic filter, glass edge absorbing filter or a solution filter. A concentrated solution of copper sulphate in water in a 1 cm thick, 2 in diameter optical cell was placed in front of the spectrometer slit to block the 800 nm gating beam. The spectrometer throughput was determined to be 3.5 times lower for horizontal polarization (corresponds to measurements with the polarizers oriented

in parallel) compared with the vertical orientation (polarizers crossed) at ~2250 cm^{-1} when probing at 400 nm. All photon counts given in this paper for measurements with the polarizers oriented in parallel ('without gate') are scaled to account for these differences.

A schematic diagram of the overall system is shown in Fig. 2. Raman scattered light was collected at 90° to the probe beam direction. A liquid nitrogen-cooled, back-illuminated CCD camera with an array 2000 × 800 pixels (Jobin Yvon) was used to record the Raman spectra. The CCD was binned vertically across 400 pixels and horizontally across 5 pixels using purpose-written software. One count corresponded to one photoelectron. The spectral resolution was ~20 and ~35 cm^{-1} with 400 and 330 nm wavelength probes, respectively. The sample solution was re-circulated in an open liquid jet of 0.5 mm diameter.

Our ps-TR^3 apparatus uses a regenerative amplifier system followed by a two-pass linear amplifier (SuperSpitfire, Spectra Physics/Positive Light) providing 800 nm, 1 ps, 2–3 mJ fundamental pulses at 1 kHz repetition rate. The fundamental laser output is split in two; 500 μJ are taken for the gating pulse to drive the Kerr gate and the remainder is frequency doubled to pump two laboratory-built optical parametric amplifiers that generate the synchronized, independently tunable pump and probe pulses required for TR^3 experiments.[17] This configuration also enables the delivery of frequency doubled, 400 nm, and tripled, ~266 nm, pulses with higher energies. For conventional ground-state Raman measurements, the pump beam is blocked.

The low-wavelength limit of the CS_2 Kerr gate is ~390 nm, determined by the electronic absorption of CS_2. The wavelength coverage extends to 700 nm where the limit is set by background due to stimulated anti-Stokes emission induced by the gating pulse in the Kerr medium. Coverage of the 300–390 nm range is facilitated by using a benzene-based Kerr gate capable of achieving a throughput of about 20%.

The overall Kerr gated TR^3 (K-TR^3) system has excellent opto-mechanical stability with only minor re-adjustment required at intervals of several days.

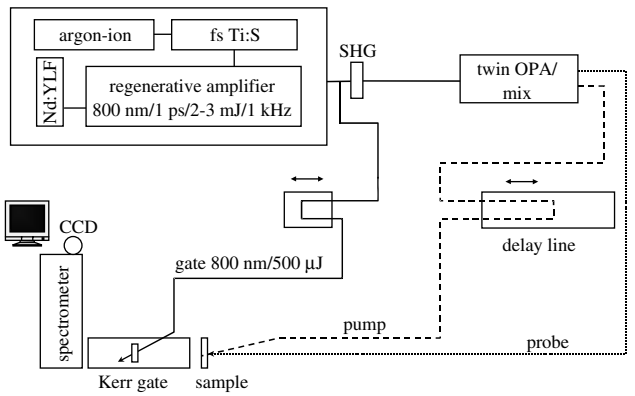

Figure 2. Schematic diagram of the picosecond time-resolved resonance Raman apparatus with the Kerr gate (K-TR^3).

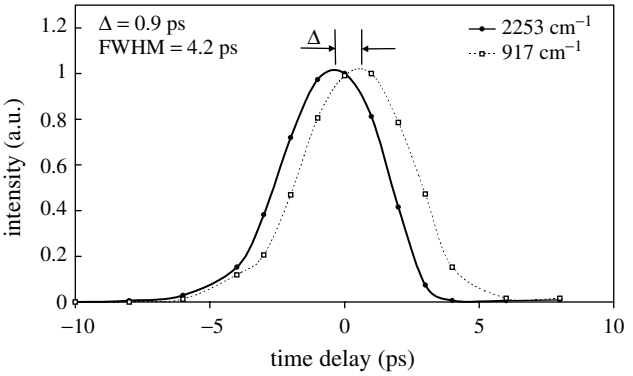

Figure 3. The intensity of the Raman signal transmitted through the gate as a function of the probe and gating pulse time delay for the 917 and 2253 cm^{-1} acetonitrile bands. The probe wavelength was 400 nm.

RESULTS

The temporal response of the gate was determined by measuring the intensity of the 2253 and 917 cm^{-1} Raman bands of acetonitrile and delaying the Raman probe pulse with respect to the gating pulse. The data were best fitted to a Gaussian profile with FWHM 4.2 ps (see Fig. 3). As expected, the curve also exhibits a small degree of asymmetry[10] due to the CS_2 relaxation time. The time lag of 0.9 ps between the two monitored Raman components is due to group velocity dispersion in the optical components. This delay is well within the opening time window of the gate and so does not present a problem in Raman measurements.

With ~500 μJ of gate pulse energy, the throughput of the Kerr gate in the open state is 40% excluding losses on optical elements, i.e. Fresnel and absorption losses (to facilitate the maximum usable spectral range, none of the optical components has an anti-reflection coating). This was determined by measuring the intensity of the 2253 cm^{-1} acetonitrile Raman line at 400 nm probe wavelength with the polarizers crossed ('with gate') and parallel ('without gate') and compensating for the polarization-dependent throughput of the spectrometer. Residual optical noise generated by the gating pulse within the Kerr cell, measured at ~415 nm, was ≤4 counts s^{-1} per CCD 5-pixel channel (about 0.1 nm). This is believed to be due to stimulated Raman scattering and its upconversion and processes leading to the onset of white-light continuum, i.e. self-phase modulation. This background was approximately constant across the detector spectral window of 40 nm. In comparison with our earlier design,[16] the new gate provides a ~2.7-times increase in Raman flux reaching the detector under the same experimental conditions. This is due to improved collection efficiency of Raman light (×1.7) and a higher overall transmission (×1.6) through the gate in the open state due to more efficient opening of the gate. This allows for correspondingly shorter acquisition times.[16] In terms of the reduction of the Raman to fluorescence ratio, it

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exhibits a similar performance for samples with fluorescence lifetimes of the order of ~1 ns. However, it substantially outperforms the earlier version for longer lived fluorescence, mainly owing to the higher extinction ratio of the polarizers (e.g. it has 10 times higher fluorescence suppression for fluorescence with lifetime 400 ns) and, most importantly, it provides a wider spectral usable range, 300–700 nm, with the higher throughput. These enhanced features represent a substantial improvement over the previous version, which had a spectral range of 630–700 nm limited by the Newport Polarcor polarizers. Although operation was possible with sheet polarizers optimized for other wavelengths, the extinction and transmission characteristics would have been substantially poorer.

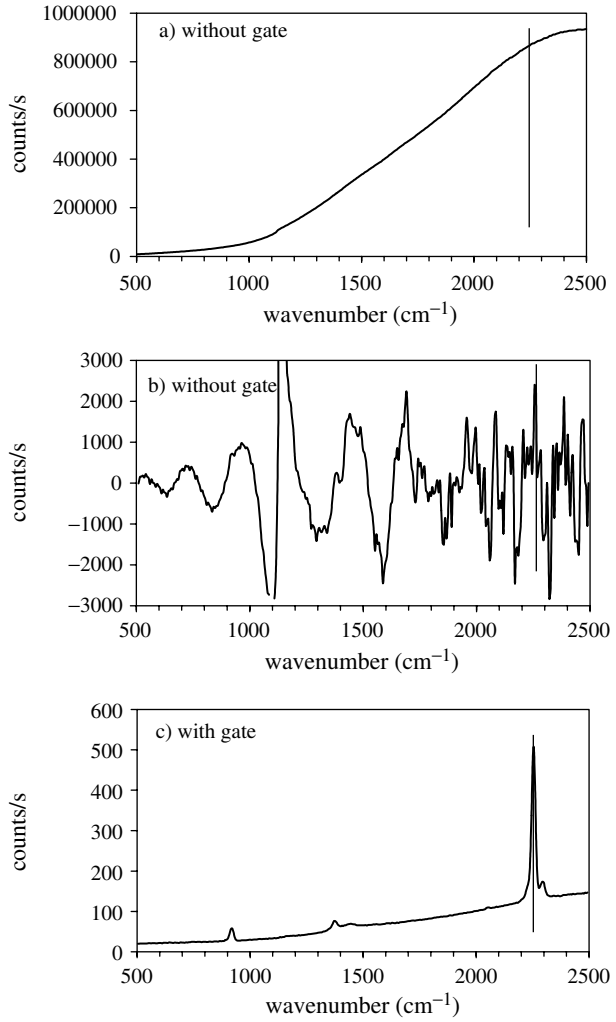

Figure 4. Raman spectra of acetonitrile contaminated with Coumarin C480 measured (a) without the gate and (c) with the gate. (b) Residuals from fitting a polynomial of 20th order to the fluorescence background shown in (a). The probe wavelength was 400 nm (1 ps, 4 μJ, 1 kHz) and the pulses were focused to a spot with diameter ~100 μm. The accumulation time was 250 s.

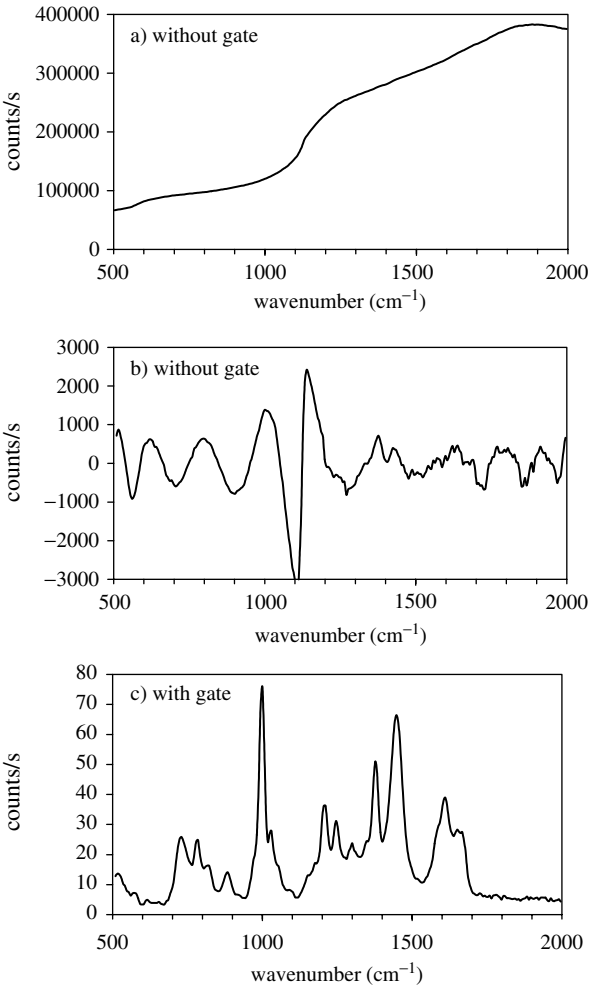

Figure 5. Raman spectra of unleaded petrol measured (a) without the gate and (c) with the gate. (b) Residuals from fitting a polynomial of 20th order to the fluorescence background shown in (a). The probe wavelength was 400 nm (1 ps, 4 μJ, 1 kHz) and the pulses were focused to a spot with diameter ~100 μm. The accumulation time was 400 s. The residual fluorescence background was subtracted by using the fluorescence-only spectrum obtained at 20 ps time delay.

To demonstrate the effectiveness of the Kerr device, we recorded Raman spectra of two chemical systems taken under extreme conditions: a well characterized solution containing the laser dye Coumarin 480 in acetonitrile and an unpurified 'industrial' sample of unleaded petrol taken directly from a motorcar petrol tank (Figs 4 and 5, respectively). The spectra obtained at 400 nm without the gate (polarizers oriented in parallel, gating pulse blocked) were in both cases dominated by a massive amount of fluorescence. An attempt to recover the buried Raman signals by fitting a polynomial background of 20th order was unsuccessful in both cases. For the dye solution, only the most intense acetonitrile band at 2253 cm^{-1} was resolved at the

level of the residual noise. However, the Raman spectrum was easily recovered with the Kerr gate suppressing the fluorescence by a factor of ~2400. The remaining sloping background is due to fluorescence passing through the gate within the 4 ps opening time of the gate and is dependent on the fluorescence lifetime, in this case is known to be 3.3 ns.[18] For the petrol solution, Raman bands were resolved from over 10 000 times more intense fluorescence background using the gate. The fluorescence lifetime was estimated to be ~30 ns from the relative intensity of the Kerr gated fluorescence recorded at +20 ps and the total fluorescence yield.

The spectrum of unleaded petrol matches well those obtained by near-infrared (NIR) Raman spectroscopy,[19] which is an alternative technique where the signal lies outside the emissive spectral range of most compounds. Our approach is instrumentally more complex and costly but it has a number of advantages, including higher sensitivity due to the $1/\lambda^4$ Raman scattering cross-section dependence, a more conveniently positioned spectral range for attaining optimum CCD detector quantum efficiency and, even more importantly, the ability to utilize the resonance Raman effect, one of the most powerful features of Raman spectroscopy. This effect offers not only a large increase in sensitivity but also an ability to enhance selectively a chemical subcomponent within the sample or the structural detail of a molecule related to a specific chromophore, or to map higher potential energy surfaces by measuring Raman excitation profiles (REP).

An example of the Kerr gate use in TR^3 spectroscopy is shown in Fig. 6. In this application, the Kerr gate was used to detect the long sought after ps-TR^3 spectrum of the highly emissive intramolecular charge transfer (ICT) state of dimethylaminobenzonitrile (DMABN).[20] The measurement has shed new light on the 40 year old problem of the nature of the anomalous fluorescence observed in polar solvents. This has been attributed by some workers to a twisted intramolecular charge-transfer (TICT) state[21–24] formed, after electronic excitation, by rotation of the dimethylamino group to a plane perpendicular to the plane of the phenyl ring. The sample was excited using a 267 nm pulse and Raman spectra of the TICT state were taken using a 330 nm pulse. This required the benzene Kerr gate. The investigation succeeded in identifying the N-ring vibrational mode by isotopic substitution of N on the amino group and provided a value for its wavenumber, 1281 cm^{-1}, which is consistent with the TICT model and disproves the PICT (planar intramolecular charge-transfer) model. The observations[20] are consistent with recent picosecond time-resolved infrared absorption measurements by Okamoto,[25] Hashimoto and Hamaguchi[26] and Chudoba *et al*.[27]

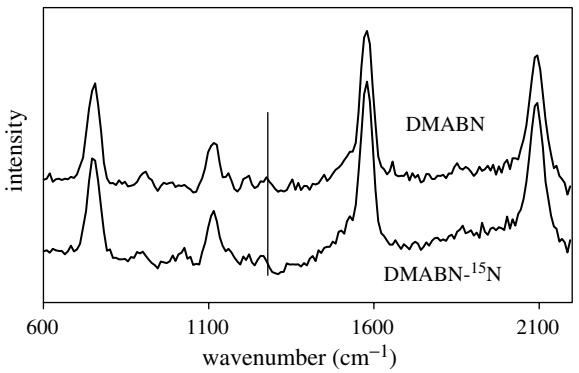

Figure 6. Picosecond TR^3 spectra of the highly emissive DMABN ICT state in methanol formed following photoexcitation to the S_1 state at time delay 50 ps. The Raman spectrum for an isotopically substituted DMABN are also shown. The pump (5–10 μJ) and probe (1–5 μJ) wavelengths were 267 and 330 nm, respectively, and the pulses were focused to a spot with diameter 100–200 μm. The accumulation time was 100 min. The DMABN concentration was $\sim 2 \times 10^{-3}$ mol dm^{-3}.

Another recent example in which the Kerr gate played a key role is a study of the interaction of the 'light switch' complex $[Ru(phen)_2dppz]^{2+}$ when directly bound to DNA.[28] This compound does not luminesce when electronically excited in aqueous solution, but the excited-state complex becomes highly emissive when it is bound to DNA. This had previously prevented using ps-TR^3 to investigate the subtle structural changes and dynamics that take place and the study of the fundamental processes responsible. The success of the recent work, we believe, paves the way for investigations of DNA–drug interactions using TR^3 and ground-state resonance Raman techniques.

The Kerr gate was also successfully used in Raman studies of highly emissive solid samples[29] and measurements of picosecond time-resolved fluorescence.[30]

CONCLUSIONS

We have developed a high-performance device, based on an optical gate with a response time of ~4 ps, for the efficient suppression of fluorescence in ground-state and time-resolved Raman spectroscopy which substantially outperforms its earlier version in terms of Raman collection efficiency, throughput and usable spectral range. The capability of the apparatus was demonstrated for ground-state Raman and ps-TR^3 spectroscopy on a range of samples which have previously been inaccessible to resonance Raman investigations because of the presence of intense fluorescence. The improved system further increases the general applicability of Raman spectroscopy and, in particular, holds great promise for industrial and biological applications where high levels of fluorescence are common.

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