Fluorescence light suppression in Raman Spectroscopy using ultrafast time gated CCD camera.

D. V. Martyshkin\textsuperscript{a}, R. C. Ahuja\textsuperscript{b}, A. Kudriavtsev\textsuperscript{a}, S. B. Mirov\textsuperscript{a}

\textsuperscript{a} Dept. of Physics, Univ. of Alabama at Birmingham, 1300 University Blvd., Birmingham, AL 35294-1178
dmartych@phy.uab.edu
Tel: (205) 934-5318
Fax: (205) 934-8042

\textsuperscript{b} LaVision GmbH, Anna Vandenhoeck Ring 19, D-37081 Goettingen, Germany

ABSTRACT

A high level of fluorescence background signal rejection was achieved for solid and powder samples by using a combination of simple low-resolution spectrograph and ultrafast intensified/gated CCD camera. The unique timing characteristics of CCD camera match exceptionally well characteristics of Ti:sapphire oscillator allowing fast gated light detection at a repetition rate of up to 110 MHz, making this approach superior in terms of duty cycle in comparison with other time-resolved Raman techniques. The achieved temporal resolution was about 150 ps under 785 nm Ti:sapphire laser excitation. At an average excitation power up to 300 mW there was no noticeable sample damage observed. The strong Hexobenzocoronane (HBC) fluorescence with a lifetime about 2.1 ns was efficiently rejected and Raman spectrum revealed. The combination of spectrometer and ultrafast gated CCD camera allows simultaneous study of spectral and temporal characteristics of emitted light for the fluorophores with a fluorescence lifetime in nanosecond range. It is particularly important in biomedical spectroscopy, since the majority of endogenous fluorophores has a relatively short lifetime of about 1-5 ns. This capability opens an exciting possibility to build a universal instrument for solving multitask problems in applied laser spectroscopy.

Keywords: Raman spectroscopy, fluorescence rejection, ultrafast gated detection.

1. INTRODUCTION

Raman spectroscopy has been used for decades as an analytical tool as well as for study of physical and chemical properties of various materials. Recent developments in laser and detector technologies allow to overcome some limitations of this method attracting substantial attention to Raman spectroscopy in research and industrial laboratories worldwide. It is proven that Raman spectroscopy could be a number one choice for many scientists and engineers due to high information content in comparison with many other methods.

As in any spectroscopic technique, Raman spectroscopy has certain drawbacks and limitations. The major disadvantage is a low (\textasciitilde 10^{-30} \text{ cm}^3) cross section of Raman scattering. Thus, Raman signal is weak comparing to the fluorescence background and is hard to detect. There are several common methods dealing with discrimination of Raman scattering from fluorescence, thermal radiation, and stray light.

The discrimination of the out-of-focus background associated with fluorescence of fluorophores distributed over sample volume and stray light is successfully realized in confocal Raman microscopy [1]. However, highly fluorescence samples are still beyond the scope of this method.

Another approach is use of UV light for sample excitation [2,3]. For the UV excitation, the Raman shifts are usually smaller than fluorescence Stokes shift of the excited molecules, permitting effective spectral discrimination of fluorescence. In addition, the majority of molecules have electronic transitions in UV region providing conditions for Resonance Raman scattering (RRS) and enhancing intensity of Raman bands up to several orders of magnitude [2,3]. However, the problems associated with samples degradation and resonance excitation of fluorescence makes this method suitable for relatively narrow class of molecules.

Finally, one could avoid fluorescence excitation by tuning laser wavelength far away from electronic transitions to near infrared region, as it is done in Fourier Transformed Raman spectroscopy (FTRS). Since Raman signal decreases as wavelength in fourth power, and some organic samples have a strong fluorescence even in NIR region, FTRS also provides relatively poor signal to noise ratio.
The utilization of simple spectroscopic systems immediately arise the problem of rejection of stray light associated with elastic scattering of laser radiation, autofluorescence of cements and coatings in optical elements, multiple reflections in optical elements and spectrometer, Raleigh scattering in samples. This problem is especially vital for the systems equipped with low-resolution spectrometers. The usual approach to reject a stray light is using a notch filter. However, the elastic scattered light can be effectively suppressed by a notch filter only if it is practically collinear to optical axis. Otherwise, scattered light can propagate though a notch filter substantially increasing background. Nevertheless, those photons have longer optical path than Raman photons and therefore are delayed.

The alternative approach is time resolved detection. It is based on utilization of pulsed excitation and gated signal detection for Raman scattering discrimination from fluorescence [4], thermal radiation [5] and stray light in time domain. The Raman scattering occurs almost instantaneously with a laser pulse while fluorescence lifetimes are in nanosecond or longer temporal range for the majority of molecules. The combination of picosecond excitation pulse with subnanosecond signal gating provides and affective rejection of the major part of the broad in time domain fluorescence signal. On the other hand, the number of Raman photons collected in one acquisition with a picosecond gate would be extremely small. Therefore the detection system should have a high duty cycle in order to obtain reasonable signal to noise ratio. Time resolved detection has been attracting a considerable attention for a few decades, however due to technological limitations it was unrealistic for the rejection of fast fluorescence until recently. A brief review can be found in Ref.[6].

Two different approaches of time-resolved detection have been shown to be effective for obtaining Raman spectra from fluorescent samples. The first method used a Kerr gate with a ~3ps resolution at 650 Hz repetition rate and allows to detect light with spectrometer-CCD detector combination [6,7]. The system achieved three orders of magnitude suppression of the background from fluorophore with a lifetime of about two nanoseconds. This method may have an average power of laser radiation only a few mW, due to a high peak power (MW), which can induce sample damage and nonlinear processes. The second method used a streak-camera and provided resolution of about 10 ps at up to 2kHz repetition rate for a fluorophore with a fluorescence lifetime four nanoseconds [8].

Due to recent advances in laser and detector technologies, the duty cycle of the detection system could be improved by several orders of magnitude. The approach we used for time-resolved detection of Raman scattering is based on combination of the state-of-the-art intensified/gated CCD camera (LaVision “PicoStar HR”), ultrafast Ti:sapphire oscillator and a simple short-focal-length spectrograph. This combination allows to achieve 150 ps temporal resolution at 76 MHz repetition rate with the average power of laser radiation increased up to 300 mW without noticeable sample damage. Utilization of this approach provided effective rejection of fluorescence and stray light background for clear crystalline samples as well as for heterogeneous (powder like) samples.

### 2. METHODOLOGY

#### 2.1 Instrumentation

The optical setup of the Raman system is shown in Fig. 1. The excitation radiation was focused on samples with a long focal length lens to a spot of approximately 400 μm through a 45-degree mirror. The scattered radiation was collected at 180 degree with respect to the excitation beam. The supper notch filter (@785nm, Kaiser Corp.) was placed inside two-lens collimator to suppress elastically scattered light.

![Fig.1. Raman system optical setup.](image)

Ti:sapphire laser (Coherent, Mira Model 900-P Laser) 785 nm radiation with a 2 ps pulse duration, 76 MHz repetition rate was used for excitation. The average power used for sample excitation was about 300 mW.
The spectrograph-detector combination consists of Acton Research “SpectroPro-150” single grating monochromator/spectrograph with a focal length of 150 mm, 1200 g/mm grating, f/4 aperture ratio, 16 cm$^{-1}$ resolution and one of two CCD cameras described below.

The PicoStar HR (LaVision) is a state of the art intensified gated/modulated CCD camera system. The image intensifier is designed for a fast gating at a repetition rate up to 110 MHz and gate width less than 200 ps, with a photocathode sensitivity in 400-900 nm range. The system equipped with a CCD chip (Interline) 1376×1040 pixels with ultra-fast readout 16 MHz pixel rate at 12 bit (or 10 frames/s) and 65% quantum efficiency (QE).

The second CCD was a conventional thermoelectric cooled TE/CCD camera (Roper Scientific/Princeton Instruments) with a mechanical shutter and 1024×256 pixels CCD chip.

2.2 Samples
Two highly fluorescent samples have been examined in this study. The crystalline CaWO$_4$ with Nd$^{3+}$ impurities was used as a transparent sample. Hexobenzocoronane (HBC) powder was used as heterogeneous sample. HBC powder was placed in fused quarts spectroscopic cell. Both samples exhibit a strong fluorescence under 785 nm radiation excitation.

3. RESULTS AND ANALYSIS

3.1 Rejection of fluorescence
The powder-like samples exhibit a strong elastic scattering of excitation radiation. The scattered light undergoes diffuse reflection causing considerable increase of out-of-focus fluorescence background and a background due to a stray light. Raman spectrum of Hexobenzocoronene (HBC) obtained with ungated CCD camera is depicted in Fig.2. The spectrum is an average of 50 acquisitions of one second each. HBC is a powder, which has a strong fluorescence even under NIR excitation. As one can see, the background signal overwhelmingly masked a weak Raman scattering signal at 1304 cm$^{-1}$.

![Figure 2](image_url)

Fig.2. Raman spectrum of HBC obtained with ungated CCD camera. The weak band at 1304 cm$^{-1}$ corresponds to HBC Raman scattering. The other bands and strong background are due to a stray light and fluorescence.

Figure 3(I,II) demonstrates time resolved light intensity at around 1178 cm$^{-1}$ and 1304 cm$^{-1}$ obtained with a gated CCD camera. The spectrum was obtained with 150 ps gate width and with 25 ps temporal step between data points. Analysis of Fig.3 shows that signal around 1304 cm$^{-1}$ is associated with a combination of HBC Raman bands and fluorescence, while signal at 1178 cm$^{-1}$ corresponds only to fluorescence. Indeed, Fig. 3,I shows that two bands have a similar behavior at a gate delays longer than 300 ps. Raman scattering could not be detected at such a long gate delays and therefore one can see fluorescence decay only. On the contrary, at a short delays, as it is seen in Fig.3,II, signal around 1304 cm$^{-1}$ rises faster than fluorescence signal around 1178 cm$^{-1}$, indicating the existing of additional Raman scattered light in the overall 1304 cm$^{-1}$ signal. The spike at 1200 ps is an artifact due to a stray light, as will be explained later in this article.

Time resolved Raman and fluorescence spectra of HBC are depicted in Fig.4. The spectra were obtained with 150 ps gate width at different gate delays. Each spectrum is an average of 50 acquisitions. Raman bands start to appear at 50 ps delay accompanied by a broad fluorescence signal. The most distinct Raman spectra were at 100-150 ps delay. Raman bands diminish completely at 300 ps delay so that one can see fluorescence only.
Fig. 3. Time resolved HBC fluorescence and Raman scattering signal at a) 1178 cm\(^{-1}\) and b) 1304 cm\(^{-1}\). The full-scale signal is shown in (I), while zoomed initial part of the same kinetics is shown in (II). The signal at a) 1178 cm\(^{-1}\) corresponds to HBC fluorescence only, while signals at b) 1304 cm\(^{-1}\) is a superposition of fluorescence and Raman scattering. Signal at 1304 cm\(^{-1}\) rises faster then signal at 1178 cm\(^{-1}\) due to additional Raman scattered light. The band at 1200 ps is an artifact due to stray light.

Fig. 4. Time resolved fluorescence and Raman spectra of HBC powder. The spectra were taken at a) 0 ps, b) 50 ps, c) 75 ps, d) 100 ps, e) 150 ps, f) 200 ps, g) 300 ps, and h) 400 ps gate delay. Each spectrum is an average of 50 acquisitions.
Figure 5 shows HBC Raman spectrum taken at 100 ps gate delay after broad fluorescence background subtraction. As one can see, we achieved an excellent signal to background ratio (>100) for a strongly fluorescent powder sample with a relatively short fluorescence lifetime was accomplished.

Study of light emission simultaneously in spectral and temporal domains of the fluorophores with a fluorescence lifetime in nanosecond range is another important capabilities of ultrafast time gated detection method. It is particularly important in biomedical spectroscopy, since the majority of endogenous fluorophores have a relatively short lifetime of about 1-5 ns. The data shown previously in Fig.3 could be used for HBC fluorescence lifetime determination. The data before 2500 ps were contaminated with a stray light, see details in the next section, therefore kinetics after 2500 ps were used for lifetime determination. The linear fit of natural logarithm of fluorescence intensity is shown in Fig.6. The time constant was found to be $-4.8 \times 10^8$ s$^{-1}$ corresponding to 2.1 ns fluorescence lifetime.

Figure 7 shows ungated Raman spectrum of CaWO$_4$ crystal contaminated with Nd$^{3+}$ ions. The stray light and luminescence background were overwhelming. Only the strongest 912 cm$^{-1}$
Fig. 7. Raman spectra of CaWO₄ crystal obtained with ungated CCD camera. The spectrum (a) is an average of 50 acquisitions of one second each. Spectrum (b) is the same as (a) multiplied by four. The 912 cm⁻¹ band corresponds to the strongest Raman band of CaWO₄ crystal. The strong 1270 cm⁻¹ band corresponds to fluorescence of Nd³⁺ (⁴F₃/2 → ⁴I₉/2) ions, which present as an impurity introduced during crystal growth process. The other bands are due to stray light inhomogeneously distributed over CCD camera surface.

Raman band of CaWO₄ corresponding to totally symmetric stretch vibrations of tetrahedrons WO₄²⁻ [9] has been resolved. The strong band at 1270 cm⁻¹ (873 nm) was due to luminescence of Nd³⁺ (⁴F₃/2 → ⁴I₉/2) [10] ions, which were present as an impurity in CaWO₄ crystal. The other bands were due to stray light inhomogeneously distributed over CCD surface. The presence of a strong background and artifact bands strongly distort Raman spectrum and make it impossible to resolve. With a time resolved signal detection, we were able to substantially improve signal to background ratio by rejecting stray light and luminescence.

Figure 8 shows the total intensity of light that reaches a detector at different time.

Fig. 8. Time resolved total light intensity obtained with gated CCD camera. The temporal step between consequent data points was 100 ps. The gate width was 200 ps. Signal at 200 ps and 1300 ps coincide with a laser pulses and correspond to Raman scattered light and elastic scattered light propagating collinear to optical axis, while strong signals at 1200ps and 1800 ps correspond to overall stray light that reached CCD camera.

The signal was taken by gated CCD camera in 100 ps temporal intervals and a gate width of about 200 ps. The spectrometer grating was positioned in such a way that Rayleigh scattered excitation radiation can be detected. The
signals at 200 ps and 13000 ps coincide with laser pulses and correspond to a combination of Raman and elastically scattered light signals. Elastically scattered light component is due to Rayleigh scattering that propagates collinearly to the optical axis through the notch filter. Strong signals at 1200ps and 14000 ps correspond to a stray light, which is a superposition of elastic scattered light in the sample and optical elements of the system, which propagate noncollinearly to the optical axis. It also includes stray light due to multiple reflections on optical elements and inside the spectrometer. One can see that stray light is delayed from Raman signal and could be effectively gated off.

Fig.9. Time resolved intensity of Raman bands of CaWO$_4$ crystal at a) 797 cm$^{-1}$, b) 912 cm$^{-1}$ and c) fluorescence band at 873 nm (1280 cm$^{-1}$) of Nd impurities in CaWO$_4$ crystal. The temporal step between consequent data points was 25 ps. The gate width was 200 ps. The temporal FWHM of Raman band is equal to 200 ps corresponding to CCD gate width. The fluorescence intensity changes of Nd are not seen, since fluorescence lifetime is substantially longer then the period of pump laser oscillations (1 ns).

Fig.10. Time resolved Raman spectra of CaWO$_4$ crystal. The spectra were taken at a) 0 ps, b) 100 ps, c) 200 ps, and d) 300 ps gate delays.

Hence, time resolved signal detection allows us an effective discrimination of Raman bands from stray light artifacts and from luminescence background. The CaWO$_4$ crystal that we used in our study has Nd ions introduced as impurity during crystal growth process. The luminescence of rare earth metal ions is usually narrow and could be easily confused with a Raman band if one uses a low-resolution spectrometer. Figure 9 demonstrates time-resolved signals at around 797 cm$^{-1}$, 912 cm$^{-1}$ and 1280 cm$^{-1}$ (873nm). The spectrum was taken with 200 ps gate width and with 25 ps temporal step between data points. The 797 cm$^{-1}$ and 912 cm$^{-1}$ correspond to Raman band of CaWO$_4$ crystal. As one can see, the signal profile has quite symmetrical shape with a temporal FWHM of about 200 ps, which match CCD camera gate width. On the
contrary, the signal around 1280 cm\(^{-1}\) (873 nm) was almost constant. This signal corresponds to luminescence of Nd\(^{3+}\) \((^4F_{3/2} \rightarrow ^4I_{9/2})\) ions, which lifetime is considerably longer (~250 µs) than the time between laser pulses (1 ns) and therefore intensity changes of Nd\(^{3+}\) luminescence could not be seen in this temporal interval.

![Figure 11](image)

Fig. 11. Stray light and fluorescence rejection from CaWO\(_4\) crystal by a time resolved light detection. The spectrum (a) was combined from several spectra at different spectrograph grating positions. Two bands at 1280 cm\(^{-1}\) and 1400 cm\(^{-1}\) correspond to Nd\(^{3+}\) \((^4F_{3/2} \rightarrow ^4I_{9/2})\) luminescence at 873 nm and 882 nm respectively. Spectrum (b) is expanded spectrum (a) in 100–600 cm\(^{-1}\) spectral region.

Time-resolved Raman spectrum of CaWO\(_4\) crystal obtained with a gated CCD camera is depicted in Fig. 10. The spectrograph grating was positioned in such a way that Nd impurities luminescence is out of detection spectral window range. The Raman spectra were acquired at different gate delays. It was strongest at 200 ps delay, which is in a good agreement with a previous time-resolved intensity profile. Several CaWO\(_4\) Raman bands at 335 cm\(^{-1}\), 405 cm\(^{-1}\), 797 cm\(^{-1}\), and 912 cm\(^{-1}\) can be clearly resolved with an excellent signal to background ratio. We were able to reconstruct a full CaWO\(_4\) Raman spectrum from several spectra obtained at 200 ps gate delay and at different spectrometer grating positions. It is shown in Fig. 11. As one can see, an excellent rejection of stray light and luminescence has been achieved.

4. CONCLUSIONS

Demonstrated time resolved Raman spectroscopy considerably improve signal to background ratio and extends capabilities of Raman measurement regarding study of samples with short florescence lifetime. We have shown that background from powder material associated with a stray light and fluorescence can be substantially suppressed even for a simple spectroscopic system equipped with a low-resolution spectrograph and gated CCD camera. The radiation of Ti:sapphire laser at 785 nm and average power up to 300 mW was used for excitation without noticeable sample damage. The high duty cycle (repetition rate 76 MHz) makes this technique superior in comparison to other time-resolved Raman methods.

Recent developments in laser and detector technologies made possible to build a simple spectroscopic system capable of solving multitask problems. Study of light emission from the variety of materials simultaneously in spectral and temporal domains opens an exciting opportunity to build a universal instrument. The combination of gated CCD camera with a confocal microscope will allow performing Raman, resonance Raman, conventional and time resolved fluorescence imaging. That kind of robust and easy in operation instrument may have extensive applications in many academic and industrial laboratories.

REFERENCES


