Andrzej KWIATKOWSKI, Janusz SMULKO, Paweł WIERZBA
GDANSK UNIVERSITY OF TECHNOLOGY, FACULTY OF ELECTRONICS, TELECOMMUNICATIONS AND INFORMATICS,
DEPARTMENT OF METROLOGY AND OPTOELECTRONICS, 11/12 Gabriela Narutowicza Street, 80-233 Gdańsk-Wrzeszcz, Poland

Raman spectra measurements for chemical identification – aspect of uncertainty sources and reduction of their effects

M.Sc. Andrzej KWIATKOWSKI

Ph.D. student at the Department of Optoelectronics and Electronic Systems, Gdansk University of Technology, Poland. His current research interests are focused on chemicals detection algorithms and methods for processing Raman spectra.

e-mail: andrzej.kwiatkowski@zak.eti.pg.gda.pl

Ph.D. Paweł WIERZBA

Research Scientist at the Department of Optoelectronics and Electronic Systems, Gdansk University of Technology, Poland. Member of SPIE and Photonics Society of Poland. His research in the field of optoelectronics covers optical measurement methods using polarization and interferometers, optical fiber sensors, applications of innovative materials in optoelectronic setups and thermal detectors. He is the author or co-author of over 100 papers.

e-mail: pwierzba@eti.pg.gda.pl

Ph.D. D.Sc. Janusz SMULKO

Assistant Professor at the Department of Optoelectronics and Electronic Systems, Gdansk University of Technology, Poland. He is a member of the board of the Polish Society of Theoretical and Applied Electrotechnics in Gdansk, a member of the Microsystems and Sensors Section of the Metrology Committee of the Polish Academy of Science and a member of the IEEE.

e-mail: jsmulko@eti.pg.gda.pl

Abstract

Raman spectrometers enable fast and non-contact identification of examined chemicals. These devices measure Raman spectra and compare with the spectra database to identify unknown and often illicit chemicals (e.g. drugs, explosives) usually without any sample preparation. Raman spectra measurements are a challenge due to noise and interferences present outside the laboratories (field applications). The design of a portable Raman spectrometer developed at the Faculty of Electronics, Telecommunications and Informatics, Gdansk University of Technology is presented. The paper outlines sources of noise and interferences (e.g. background removal, spectra smoothing) contaminating Raman spectra measurements as well as signal processing techniques required to reduce their influence. The authors consider influence of packages (glass or plastic vials) on effectiveness of detection of selected chemical mixtures and they propose a method that reduces this factor. Next, the impact of acquisition time and external lighting on the accuracy of Raman spectra measurements is investigated. The synchronic detection is introduced to diminish changes of outside sample irradiation. Finally, the etaloning error present in the measured spectra is considered and attenuated by an additional procedure.

Keywords: Raman phenomenon, Raman spectroscopy, noise, interference, etaloning effect.

Problemy pomiarów widm Ramana podczas identyfikacji substancji chemicznych

Streszczenie

Spektroskopy Ramana umożliwiają szybką i bezkontaktową identyfikację badanej substancji chemicznej. Mierzą widma Ramana i porównują z biblioteką widm, aby identyfikować nieznaną, często nielegalną substancję chemiczną (np. narkotyki, materiały wybuchowe) bez potrzeby przygotowania badanej próbki. Pomiar widm Ramana w warunkach polowych jest dużym wyzwaniem ze względu na obecność wielu źródeł szumów i zakłóceń, m.in. szumy przetworników CCD, fluorescencja, szumy kosmiczne, oświetlenie zewnętrzne. W pracy przedstawiono projekt przenośnego spektrometru Ramana opracowanego na Wydziale Elektroniki, Telekomunikacji i Informatyki Politechniki Gdańskiej. Opisano źródła szumów i zakłóceń obecnych podczas pomiarów widm Ramana oraz techniki przetwarzania sygnałów zmniejszające ich wpływ (np. usuwanie tła, widm wygładzanie). Autorzy rozważają także wpływ opakowań (szklanych lub plastikowych) na efektywność detekcji wybranych mieszanin chemicznych i proponują metodę, pozwalającą na jego redukcję. Następnie przedstawiono zależności między czasem ekspozycji a dokładnością pomiarów widm Ramana. Określono skutki oddziaływania zewnętrznego (niepożądanego) oświetlenia na jakość pomiarów oraz przedstawiono sposób ich ograniczenia przy użyciu pomiarów synchronicznych. Następnie opisano zjawisko etaloningu oraz procedurę jego usunięcia w kontrolowanych warunkach pomiarowych, za pomocą dodatkowych procedur.

Słowa kluczowe: zjawisko Ramana, spektroskopia Ramana, szumy, zakłócenia, etaloning.

1. Introduction

Chemical detection, based on measurements of its Raman spectra, uses a comparison of a registered spectrum with the reference spectra stored in a database. This database is often created in the laboratory environment applying precision Raman spectrometers with high resolution. Field measurements of spectra of the inspected substances are usually made by less accurate devices.

The measurements of Raman spectra are interfered. The main sources of interference are: fluorescence induced by the laser beam, cosmic radiation, external light sources, noises of the CCD detector and applied charge amplifiers and the etaloning effect [1]. The impact of some of these sources can be reduced by designing an appropriate measurement path, or by using dedicated correction algorithms. This paper describes practical problems encountered by the project team during the design of a portable Raman spectrometer. The portable Raman spectrometer was designed and built at the Department of Optoelectronics and Electronic Systems of Faculty of Electronics, Telecommunications and Informatics, Gdansk University of Technology.

Firstly, the results of external light source attenuation by synchronous detection are presented. Secondly, influence of the exposure time on the efficiency of interference attenuation in both modes (synchronous and continuous) is considered. Thirdly, a method for diminishing etaloning effects is described. Finally, the similar method was applied to remove the impact of a package of the inspected chemicals on the registered Raman spectra.

2. External interference attenuation

Field measurements of Raman spectra are performed in the presence of external illumination sources (solar light, artificial light in buildings). The spectrum intensity of this light can be much stronger than the recorded Raman spectrum of the investigated chemicals, which makes their identification almost impossible. Therefore, it is recommended to take the measurements in darkness. Unfortunately, it is often not possible. In that case, the authors decided to apply synchronous detection.

The method validity and its resistance to external illumination was confirmed by measurements of diamond Raman spectra in synchronous and continuous modes using a laser emitting at 785 nm. The laser power was set to 70 mW and the exposition time was 30 s. To simulate conditions of field measurements, the process was carried out in presence of a strong ambient light source of a bulb. The results are shown in Fig. 1.

From Fig. 1 it can be concluded that the continuous measurement is more sensitive to external lighting (dashed line in Fig.1). Along the spectrum a high background level due to a broadband spectrum of the applied bulb is observed. In the case of synchronous measurements the background spectrum was strongly attenuated. A ratio of the main spectral line level (around 1330 cm^{-1}) to the background level was significantly improved, thus enhancing the correct estimation of the relative amplitude and half-width (FWHM) spectrum of the diamond line [2].

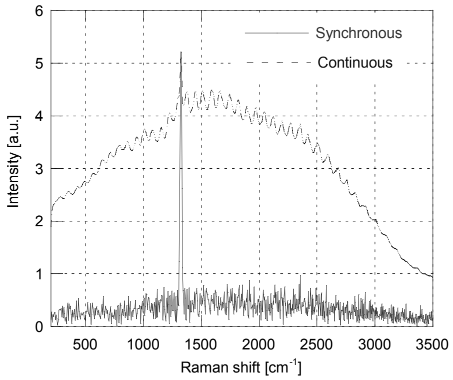

Fig. 1. Raman spectrum of diamond under conditions of strong external illumination: continuous mode (dotted line), synchronous mode (solid line)

Rys. 1. Widmo Ramana diamentu zarejestrowane w warunkach silnego oświetlenia zewnętrznego: pomiar ciągły (linia przerywana), pomiar synchroniczny (linia ciągła)

The synchronous detection takes into account the components of the spectrum whose intensity depends on the laser illuminating the inspected sample by a modulated beam. The external lighting is uncorrelated with the excitation beam and is reduced by applying the synchronous detection algorithm presented in detail in literature [3].

3. Influence of the exposure time on the measurement quality

Effect of the exposure time on the measurement quality in continuous mode was tested using the Raman spectra of diamond using 785 nm laser at 70 mW optical power at different exposure times (Table 1). It was observed that in the case of continuous measurements an increase in the exposure time caused an increase of the amplitude of the main spectrum line and the background level. It can be easily explained by the fact that the output signal of a CCD is directly proportional to the number of photons detected during the exposure time. Therefore, this feature should be taken into account when choosing the exposure time. If a substance gives a strong Raman signal, the measurement time should be chosen shorter so as not to overload the CCD. The two indicators for the estimated spectra were calculated. The first one was the ratio of the maximum value of the main diamond spectrum line to the average value of the spectrum (Table 1). The second indicator was the ratio of the maximum value of the main diamond spectrum line to the standard deviation of the spectrum (Table 2). Additionally, all spectra were subjected to a pre-processing procedure (background removal, smoothing), described in detail in [2].

Tab. 1. Maximum value of the main spectrum line to the average spectrum value ratio

Tab. 1. Stosunek wartości maksymalnej linii widmowej do wartości średniej widma

Time [s]	Before pre-processing	After pre-processing
10	2,19	65,73
20	3,10	67,60
30	3,81	68,62
40	4,30	66,65
60	5,15	67,35
80	5,75	67,66
100	6,21	67,63
120	6,56	67,69

Tab. 2. Maximum value of the main spectrum line to the standard deviation ratio

Tab. 2. Stosunek wartości maksymalnej linii widmowej do odchylenia standardowego widma

Time [s]	Before pre-processing	After pre-processing
10	23,78	16,17
20	19,23	16,25
30	17,79	16,26
40	16,94	16,14
60	16,25	16,16
80	15,91	16,18
100	15,72	16,19
120	15,61	16,20

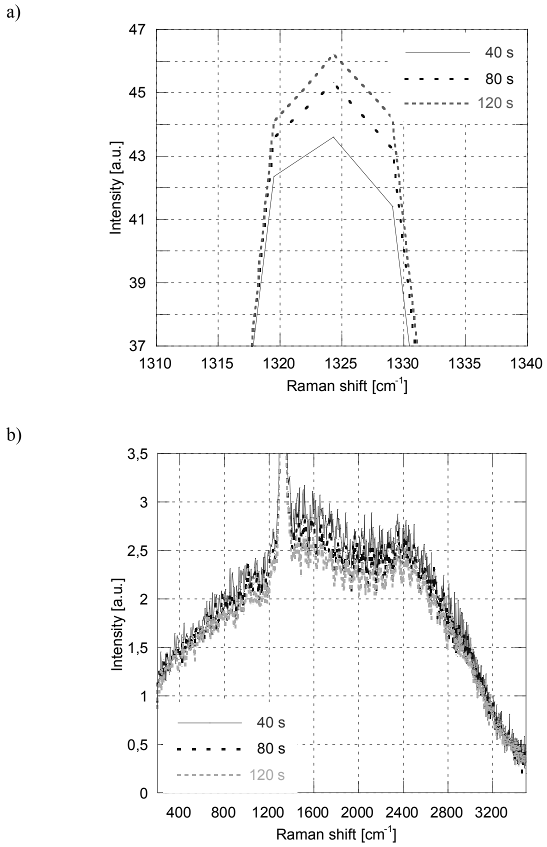

Fig. 2. Effect of the exposing time for synchronous measurement of diamond spectra: a) the level of maximum spectral line, b) the level of background

Rys. 2. Wpływ długości czasu pomiaru synchronicznego na: a) poziom wartości maksymalnej linii widmowej b) poziom tła

According to the obtained results it should be noted that an effective procedure for background removal allows significantly decreasing the exposure time. After background removal the amplitude of the main diamond spectral line to the average value of spectrum ratio and the amplitude of the main diamond spectral line the to the standard deviation of spectrum ratio remained unchanged within a large range of the exposing time (10÷120 s) in continuous mode. It may be assumed that the background removal can reduce the exposure time necessary to estimate properly the Raman spectrum.

Another experiment was carried out to determine the effect of the estimate time on the background spectrum intensity in synchronous mode. For that purpose, the spectra of diamond acquired for different exposure times were estimated. Measurements were taken at frequency modulation of 1 Hz and laser optical power 70 mW. The results of the experiment are presented in Fig. 2. It can be concluded, that the amplitude of the diamond spectrum line is directly proportional to the exposure time. Increase in the exposure time results in increase in the amplitude of the main spectrum line (Fig. 2a) and simultaneously in decrease in the background level (Fig. 2b), as expected from the theory of synchronous detection [3].

4. Etaloning effect reduction

The etaloning effect appears as unwanted characteristic fringes visible on the spectrum background. These fringes look like an amplitude modulated sinusoidal function (Fig. 3) and can be wrongly identified as spectral lines by the detection algorithm [4]. Etaloning effect is caused by reflections inside the CCD converter. The beams reflected from the converter internal layers interfere with the original beams falling on the converter surface. It results in light intensity suppression in some places (destructive interference), and amplification (constructive interference) in others (Fig. 3).

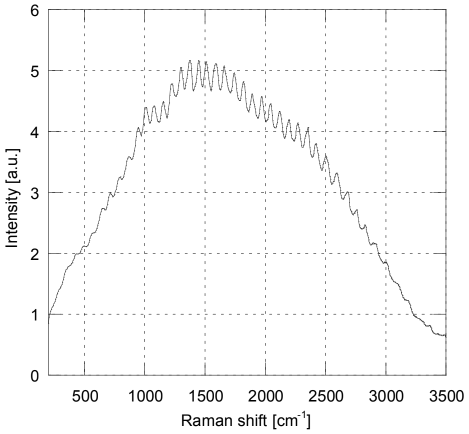

Fig. 3. The spectrum of white light (of bulb) with visible etaloning effect

Rys. 3. Widmo światła białego (żarówki) z uwidocznionym efektem etaloningu

This phenomenon is difficult to eliminate by algorithmic methods. There are some correction algorithms which allow removing unwanted fringes, but they work only under strictly controlled measurement conditions [5, 6].

It was observed that the intensity and shape of the etaloning effect depended on CCD lighting conditions which were an excitation laser beam, external lighting and the sample fluorescence. This phenomenon is especially troublesome in the cases when the Raman spectra exhibit spectral lines of low intensities (e.g., Raman spectra of traces of drugs in saliva) which are masked by the detrimental etaloning effect.

Measurements of trace amounts of chemicals are performed by using the Surface Enhanced Raman Spectroscopy (SERS) [7]. This technique, relying on multiple signal reflections from metallic surfaces, provides a significant gain of the Raman signal in comparison to classical measurements. SERS measurements are made by placing the investigated substance on a special plate or gel with metallic drops (usually silver or gold).

The spectrum can be corrected by removing the etaloning effect with two different measurements. First one when the spectrum of pure SERS plate is measured (Fig. 4 - solid line). Then a drop of the investigated liquid sample is put on the surface of SERS plate and the second measurement is made (Fig.5 - dotted line).

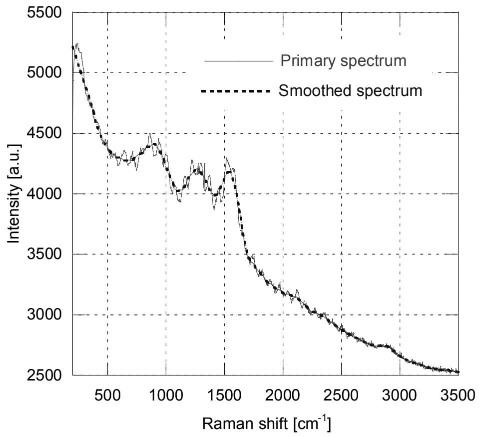

Fig. 4. The spectrum of SERS substrates: the estimated spectrum (continuous line), after procedure of smoothing (dotted line)

Rys. 4. Widmo podłoża SERS: pomierzone (linia ciągła), po poddaniu operacji wygładzenia (linia przerywana)

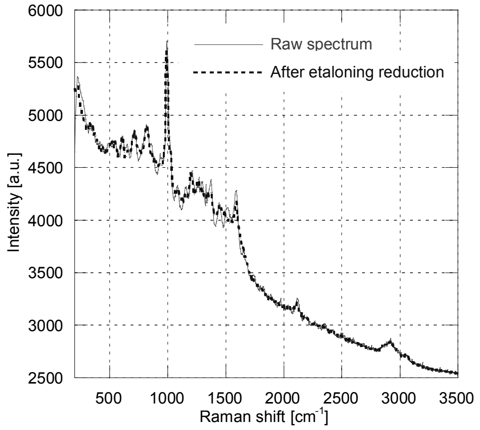

Fig. 5. Amphetamine sulphate 1mg/ml; spectrum obtained by applying SERS technique

Rys. 5. Siarczan amfetaminy 1mg/ml; widmo otrzymane techniką SERS

The next step is to define the amplitude of etaloning waving in the background. For this purpose, the pure SERS plate spectrum is subjected to filtration to get a smoother spectrum. A good solution is a Savizky-Golay filter with a window wider than the number of samples per one waving period. Such smoothing reduces unwanted fringes effectively while maintaining the spectrum trends (Fig. 4 – dashed line.). Next, the vector of difference between the original and smoothed spectrum is calculated. In this way we can get a vector of the wave caused by etaloning effect. Knowing this vector, the corrected spectrum of the investigated substance can be calculated according to the proposed formula:

$$os[i] = ms[i]\cdot\frac{sg[i]}{sg[i]+r[i]}\,,$$
$$r[i] = e[i] - sg[i]\,. \tag{1}$$

where: os – spectrum of the substance after correction, ms – spectrum of the substances before correction, sg – the smoothed substrate spectrum, r – etaloning vector, e – spectrum of SERS plate with etaloning, i – spectrum sample's index.

The result of the algorithm for a sample of amphetamine sulphate (1 mg/ml) is shown in Fig. 5. The spectrum is smoothed by reducing the waves caused by etaloning effects. Therefore chemical detection by Raman spectra is more efficient [2].

5. Interference of packaging

It is usually assumed that Raman spectroscopy enables chemical detection of substances that are contained in the package without opening. It is known that some packages have so weak spectral lines that do not interfere in a significant way with the spectrum of the examined substance.

However, according to numerous measurement results the impact of chemicals packaging (bottles, tubes, etc.) can be significant. Fig. 6 shows the ethanol spectrum measured through a glass vial, the pure ethanol spectrum l, and the glass vial spectrum. All spectra were normalized to the maximum value.

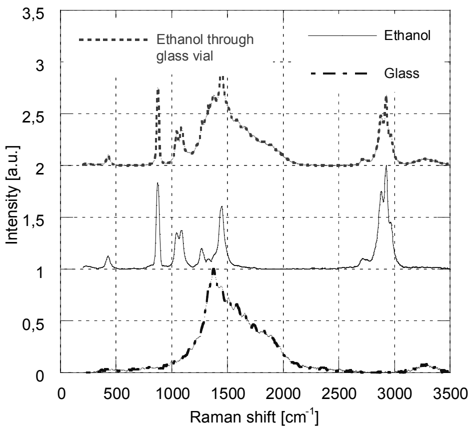

Fig. 6. Influence of packaging on Raman spectrum of the investigated chemical; the spectra are shifted by a constant value to illustrate their differences

Rys. 6. Wpływ opakowania na widmo badanej substancji; widma są przesunięte między sobą o stałą wartość aby zilustrować różnice

Analyzing Fig. 6 it should be noted that in the range 1200÷2200 cm^{-1} and above 3200 cm^{-1} (top line) the glass spectrum (bottom line) is intense and has detrimental effect on the measurement quality of ethanol spectral lines. To eliminate this effect, a simple procedure requiring two measurements is proposed.

The first one is the measurement of Raman spectra of the package. The index and value of maximum sample are recorded. Then the spectrum of chemicals together with the packaging (e.g. ethanol in a glass bottle) is measured. Following, the scaled spectrum of the package is subtracted from the second spectrum.

A scaling ratio is obtained by dividing the recorded value by the value of the second spectrum sample (investigated chemical inside package) which has the same index as the earlier recorded one. The result of the algorithm is presented in the Fig. 7.

The proposed algorithm can fail when the pick value of the packaging spectrum coincides with the pick value of the investigated chemical spectral lines.

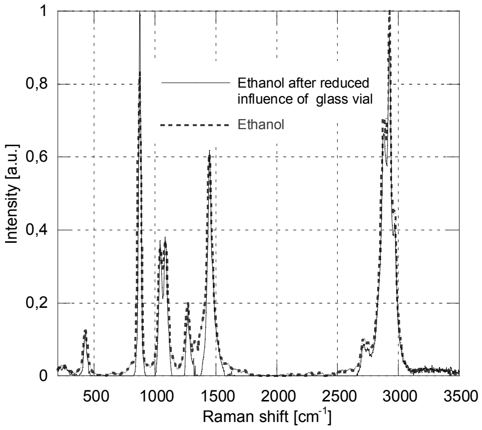

Fig. 7. The result of the packaging removal algorithm

Rys. 7. Wynik działania algorytmu usuwania opakowania

6. Conclusions

This paper presents practical problems of Raman spectroscopy which emerged during use of a portable Raman spectrometer. Attempts were made to solve them. Interference of external lighting can be attenuated by synchronous detection. Additional procedure of Raman spectra smoothing and background removal can ensure the short exposure time. The supplementary measurement of package Raman spectrum can reduce its influence on chemical detection. The etaloning effect can be reduced by smoothing procedure as well.

This research was financed by the Polish Ministry of Science and Higher Education (2009/2011), developmental project no. OR 0000 2907. and DS funding of the Faculty of Electronics, Telecommunication and Informatics.

7. References

[1] Bielecki Z., Rogalski A.: Detekcja sygnałów optycznych, WNT, Warszawa, 2001.

[2] Kwiatkowski A., Gnyba M. Smulko J.: Algorithms of chemicals detection using Raman spectra, Metrology and Measurement Systems, Vol. XVII (2010), No. 4, pp. 549–560.

[3] Kotarski M., Smulko J.: Assessment of synchronic detection at low frequencies through dsp-based board and pc sound card, XIX IMEKO World Congress, September 6-11, 2009, Lisbon, Portugal, pp. 960–963.

[4] Mário Augusto da Silva Martins, Dayana Gonçalves Ribeiro: Shifted-excitation Raman difference spectroscopy for in vitro and in vivo biological samples analysis, Biomedical Optics Express, Vol. 1 Issue 2, pp.617-626, 2010.

[5] McCreery R.L.: Raman Spectroscopy for Chemical Analysis, John Wiley & Sons, New York 2000.

[6] Fochesatto J., Sloan J.: Signal processing of multicomponent raman spectra of particulate matter. Selected topics in electronics and systems, Vol. 49, pp. 49–66, World Scientific 2009.

[7] Blackie E.J., Le Ru E.C., Etchegoin P.G.: Single-Molecule Surface-Enhanced Raman Spectroscopy of Nonresonant Molecules, J. Am. Chem. Soc., Vol. 40, pp. 14466–14472, 2009.

otrzymano / received: 16.09.2011
przyjęto do druku / accepted: 02.11.2011 *artykuł recenzowany*