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# Analysis of the alteration in the optical configuration of Raman spectrometer: Optimization of signal-to-noise ratio (SNR) in a specific wavelength range of clinical interest

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**Abstract.** The present article is focused on the optimization of the optical parameters of a Raman spectrometer in order to obtain a minimum width of its spectral lines. In this way, using as reference the width of a fingerprint band of a calcified biological tissue, a spectral line without distortion or any loss of resolution was identified. This optimization is employed with the aim of improvement of the signal-to-noise ratio (SNR). A great improvement in the efficiency of the spectral collect was obtained, which can reduce significantly the time of diagnosis of target tissues, such as the calcified coronarian tissue. Therefore, the potential application of this new spectroscopic system increases the efficiency and precision, favoring the security of this technique to future *in vivo* applications. The excellent results obtained in this work become this spectroscopic system a powerful tool to the clinical diagnosis of several diseases.

Keywords: Raman spectroscopy, optimum wavelength, signal-to-noise ratio (SNR), biomedical instrumentation, atherosclerosis

## 1. Introduction

Raman spectroscopy is an important technique that permits to obtain precise information about biochemical features from the biological sample, representing a potential use for diagnostic and therapy medical applications [1,2]. For this kind of application, it is necessary an excellent system of spectral collection, which propitiates an adequate identification of the spectral bands obtained in several spectroscopic techniques, such as Raman spectroscopy (RS).

Several methodologies have been employed in order to improve the resolution of the Raman spectra, especially focusing its biomedical applications [3–5]. The most common procedures associated to this work of improvement of the spectral resolution in Raman spectroscopy are basically correlated to the increase in the power of the laser used to irradiate the sample, increase in the time of exposition in the

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Charge Coupled Device (CCD) detector and light intensity increase that get into at the camera through the slit of the optical window.

In the first case, the increase in power of the laser is not a trivial procedure due to the high cost of specific lasers with elevated power, which is not available to several laboratories [6–9]. In the second case, the increase of time of exposition generates a long process of data collection, which would not be adequate, especially regarding to clinical applications, where the rapid diagnosis is necessary due to the state of risk inherent to the patient. Indeed, it is interesting that the diagnosis can be made in a reduced interval of time to facilitate the posterior steps of the therapy [10–12]. In the last case, the proposal is to increase the width of optical slit of the spectrometer to improve the intensity of the spectral signal. This signal increase should not be associated to any loss of spectral resolution, implying that an optimum spectral condition must be developed. Thus, it is interesting to increase the slit associated to the wavelength of a specific band, until an optimum point, where is obtained a maximum signal-to-noise ratio (SNR).

In the present work, we are proposing a simple and accessible methodology to several laboratories to obtain maximum signal intensity without damage to the spectral resolution. In fact, this approach, which is based in the selection of a specific wavelength for each sample, has not been found in the literature.

The choice of this more adequate wavelength to the slit is dependent of the specific sample analyzed by Raman spectroscopy, since for each biological tissue there are specific molecules with their typical vibrational bands. In this way, it must be chosen the more intense and representative peak encountered in a determined sample to develop this spectroscopic analysis in order to maximize the intensity of the respective peak signal. For instance, we can mention the fingerprint peak of mineralized tissues that is found approximately in 960 $cm^{-1}$, which is associated to the hydroxyapatite [13]. On the other hand, studying atherosclerosis the peculiar band related to the cholesterol, which is found around 1445 $cm^{-1}$, would be more suited to the obtaining of an excellent SNR [14,15]. Indeed, we can propose this methodology of diagnosis for various pathological states, depending of the molecules present in each sample.

Thus, in order to apply Raman spectroscopy in biochemical analysis and diagnosis, this new optical system would be very interesting, since the sensitivity and the resolution of this technique would be significantly increased. Several modern applications of this tool would be intensely improved, especially regarding the employment in biomedical sciences. It is important to notice that one of the main focuses of the present research in the biomedical area is related to the obtaining of non-invasive or, at least, minimally invasive methods of biochemical analysis and diagnosis. Indeed, the clinical process to access pathological changes in tissue is currently related to the histopathology. However, the management of biopsy material and the interpretation of the respective analysis are not trivial procedures. The clinical characterization based on these analyses inevitably leads to diagnostic delay and the added possibility of taking an unrepresentative sample. Furthermore, this kind of clinical procedure presents high cost and provokes significant patient trauma [16]. In this way, the research focused on the improvement of the resolution of Raman spectrum is relevant, since it allows more complex applications of this tool, such as biochemical analysis and diagnosis.

Therefore, the present article can be a very relevant contribution to the clinical methods of analysis as well as the quality of life for patients with different diseases.

## 2. Materials and methods

The spectrometer employed in the spectral analysis of this work is HoloSpec, model HS-f/1.8i-NIR with grade of holographic dispersion VPT™ of Kaiser Optical system. This system is analyzed by a dif-

fraction grating with a wavelength range between 400 and 1000 nm, furnishing a maximum efficiency in the wavelength selected in the respective measurement. In this equipment, the minimum value of the slit is 25 μm and the maximum value is 3 mm. The holographic diffraction array "Raman gratings" used in this work is optimized to the wavelength of excitation of 830 nm (HSG – 830 LF). The value of linear dispersion "$d$" presented by this model to the grade of holographic diffraction (Low Frequency Stokes Grating) is 4.9 nm/mm to 830 nm, which is observed to "Low Frequency Stokes gratings" from 400 until 1860 $\mathrm{cm}^{-1}$ [17].

The numeric aperture (NA) undergoes variations in the aperture parameter of the slit from 100 until 300 μm, where is observed the specific behavior associated to very thin width of band, which is especially appropriate to Raman spectroscopy. The sample used in the present work is a calcified tissue of coronary of human heart, where the spectral band of interest is the line of hydroxyapatite (960 $\mathrm{cm}^{-1}$) [18,19].

Considering the elementary equation [20]:

$$\nu = \frac{1}{\lambda},$$

where $\nu$ represents wavenumber in $\mathrm{cm}^{-1}$ and $\lambda$ corresponds to the wavelength of the radiation used in the respective analysis, we can obtain through mathematical derivation the subsequent equation:

$$\mathrm{d}\nu = \frac{1}{\lambda^2}\,\mathrm{d}\lambda \quad \text{implying that} \quad \Delta\nu = \frac{1}{\lambda^2}\Delta\lambda.$$

Therefore, $\Delta\nu$ represents the minimum slit of the spectral peak in $\mathrm{cm}^{-1}$ and $\Delta\lambda$ corresponds to the minimum resolution that the system to detect in nanometers [17] (1):

$$\Delta\lambda = d \cdot D, \tag{1}$$

where $d$ represents the linear dispersion from diffraction grating that belongs to the spectrometer and $D$ corresponds to the slit aperture as can be observed in Eq. (1). The manufacturer of the diffraction grating [17] shows the linear dispersion as function of the laser wavelength in agreement with the subsequent Table 1.

From these data, we can calculate any linear dispersion using other wavelengths by linear regression utilizing the subsequent expression:

$$f(\lambda) = a \cdot \lambda + b. \tag{2}$$

Allowing to infer that the Eq. (2) can be represented according with the proposal demonstrated in Eq. (3):

$$d = 0.006 \cdot \lambda - 0.1469. \tag{3}$$

The spectrometer is constituted by a diffraction grating, which shows the minimum spectral peak slit that the equipment can resolve in $\mathrm{cm}^{-1}$.

Table 1
Raman gratings that specifies the linear dispersion as function of wavelength of the laser

| Wavelength of the laser $\lambda$ (nm) | 488 | 514.5 | 532 | 632.8 | 647 | 752 | 785 | 830 |
|---|---|---|---|---|---|---|---|---|
| Linear dispersion $d$ (nm/mm) | 2.8 | 3.0 | 3.1 | 3.6 | 3.7 | 4.4 | 4.6 | 4.9 |

The minimum width of the spectral line, which is resolved by Raman spectrometer, is measured in $cm^{-1}$, and is associated to $\Delta\nu$ and demonstrated by Eq. (4):

$$\Delta\nu = \frac{1}{\lambda^2} \cdot dD, \tag{4}$$

where $d$ is the linear dispersion, $D$ is the aperture of slit and $\lambda$ is the wavelength of the laser of excitation [21]. The value of $\Delta\nu$ to the respective system, to all wavelengths collected through RS, must to permit the identification of a determined spectral band, which must to present higher signal-to-noise ratio (SNR).

## 3. Results

The Raman spectrum of calcified tissue from human heart is shown in Fig. 1. The main peak of this spectrum corresponds to the spectral band associated to the hydroxyapatite, which is centered in approximately 960 $cm^{-1}$ [13] with a width of band FWHM (Full Width at Half Maximum) of 26 $cm^{-1}$. In this way, this value corresponds to the maximum width of spectral band that the system should present, i.e., the value of $\Delta\nu$ must be equal or lower than 26 $cm^{-1}$ in order to obtain a highest resolution that the Raman spectroscopy can reach in this spectrum.

Therefore, analyzing the Raman spectrum with the principal band in 960 $cm^{-1}$, varying the width of slit from 100 to 300 μm, it was observed changes in the $\Delta\nu$ values as function of the aperture of the slit. To the slit with 100 μm, the value of $\Delta\nu$ calculated using the Eq. (1) is 6.5 $cm^{-1}$, but if the aperture of the slit is altered to 200 μm the value of $\Delta\nu$ increases to 12.7 $cm^{-1}$. Subsequently, fitting the slit to 300 μm the value of $\Delta\nu$ corresponds to 19.5 $cm^{-1}$, which is still lower than the maximum width that is 26 $cm^{-1}$, maintaining, in this way, the high resolution of the measurement. Consequently, it is observed that the increase in the width of the slit resulted in a more elevated value of $\Delta\nu$, keeping the same value to the Raman shift (960 $cm^{-1}$).

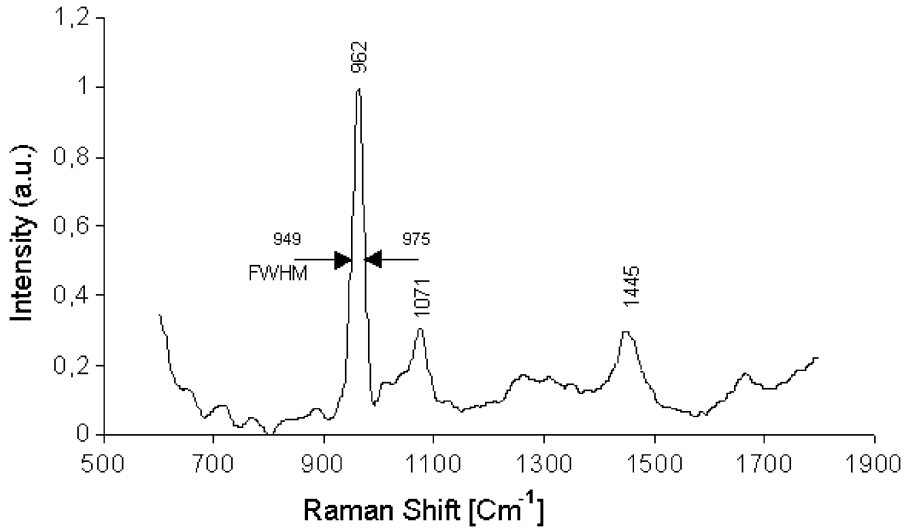


Fig. 1. Raman spectrum of coronarian artery, presenting the typical spectral band of hydroxyapatite.

On the other hand, the increment in the $\Delta\nu$ value generates a decrease in the spectral resolution, due to the increase in the broadness of peaks. In this way, these broader spectral lines overlapped the spectral lines of lower intensity and width. It is important to notice that these small peaks can be decisive to the development of an adequate spectral analysis. Actually, a more detailed and informative spectral evaluation regarding physico-chemical properties of the sample could be obtained with the identification of these other peaks. However, the aperture of the slit could be adjusted specifically to each peak of interest, without to loss resolution. Therefore, this new technique presented in this article can be very useful to obtain this optimum resolution, which allows the identification of the important peaks, which are isolated in each analysis.

Figure 2 demonstrates the significant difference between the normal and calcified tissues, illustrating the analytical potential of Raman spectroscopy, which would be accentuated with a representative improvement of the SNR.

In Fig. 3, it is observed clearly that the $\Delta\nu$ value decreases with the decrease of the aperture of the slit, propitiating a higher capacity of resolution to the technique and, consequently, permitting a more

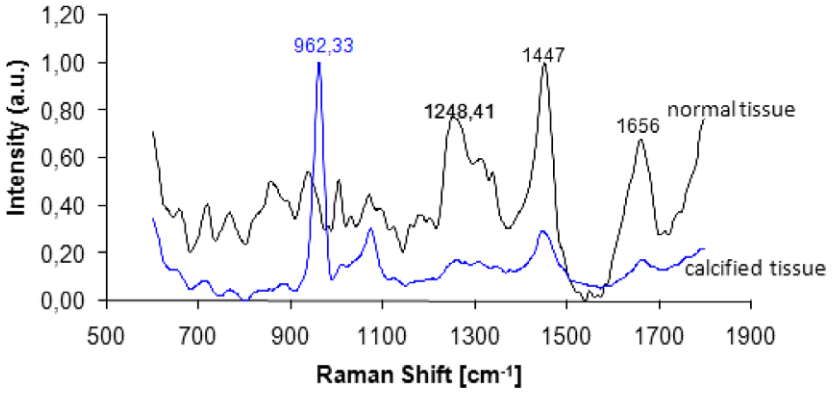


Fig. 2. Raman spectra of normal and calcified coronarian tissues without spectroscopic alterations, denoting the significant difference of intensity, and even of wavelength, between the typical fingerprint bands of each spectrum.

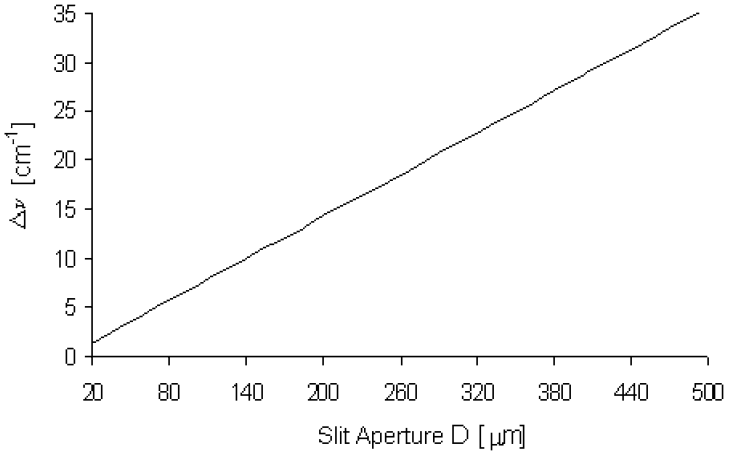


Fig. 3. $\Delta\nu$ values as function of the aperture of slit in the wavelength of 830 nm, with an interval of variation in slit from 20 until 500 μm.

precise identification of the peaks of interest that are encountered in the spectrum.

Considering the variation in the light power that inside in the camera of CCD and the resolution of the Raman spectrometer $\Delta_{CSP}$, which corresponds to the variation in the "capability of spectral resolution" of the equipment, it was possible to determine the spectral behavior in each case. Indeed, employing Eq. (5), we can evaluate the spectral tendencies described previously. Therefore, it is possible to determine the $\Delta_{CSP}$, as the inverse of minimum width of the spectral line that the Raman spectroscopy can resolve ($\Delta\nu$) [22].

Therefore: $\Delta_{CPS} = \frac{1}{\Delta\nu}$.

$$\frac{1}{\Delta\nu} = \frac{1}{(1/\lambda_R^2)D(0.006\lambda_R - 0.1469)}. \tag{5}$$

The relationship demonstrated above is presented in Fig. 4. Considering the previous discussion, it is necessary to employ the highest value of $\Delta\nu$ that still permits to obtain a spectral peak with significant resolution. This can be observed in Fig. 4, that permits to note the relationship between the power transmitted with the signal-to-noise ratio (SNR). Indeed, an elevation in the light potency transmitted increases concomitantly the signal-to-noise ratio (SNR) of a directly proportional way.

Considering the spectral peak of the calcified tissue presented in Fig. 1, it seems to be very adequate the use of 26 $cm^{-1}$ to $\Delta\nu$ ($\Delta_{CPS} \sim 0.038$ cm), which is generated by an aperture of slit of 367 μm. This slit corresponds approximately to 300 μW of light potency transmitted (Fig. 4). However, to an aperture of slit of 200 μm, it is possible to verify the value of $\Delta\nu$ around 14 $cm^{-1}$, which corresponds to $\Delta_{CPS} \sim 0.071$ cm. It is important to register that this value is obtained with a power of 133 μW (Fig. 3). Employing a value of 26 $cm^{-1}$ to $\Delta\nu$, the ratio of power is increased in approximately 2.25 when compared with the ratio of power encountered with $\Delta\nu$ of 14 $cm^{-1}$, denoting a significant improvement in the signal-to-noise ratio (SNR) of the spectrum.

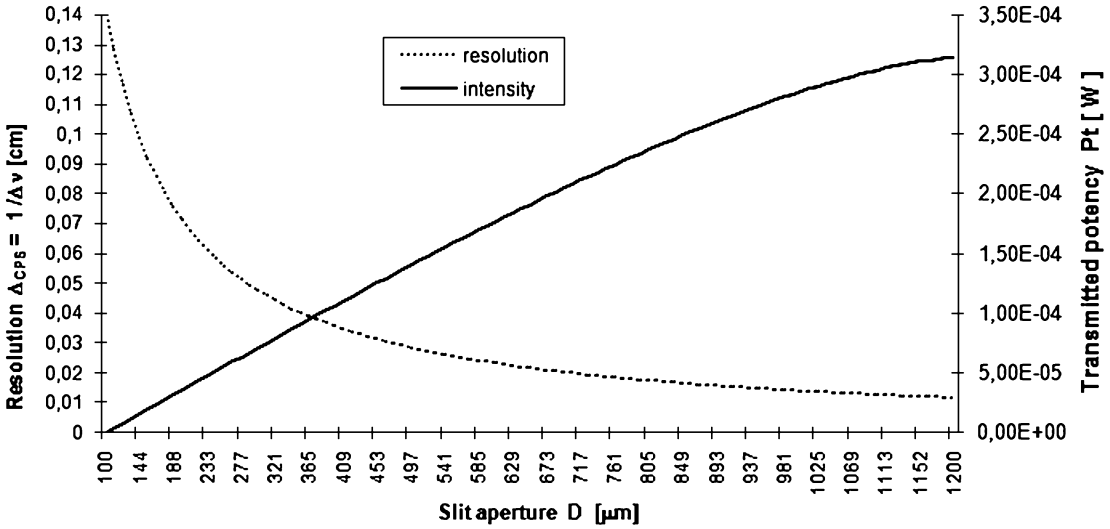


Fig. 4. Relationship between variation of "capability of spectral resolution" ($\Delta_{CPS}$) and light power transmitted through the aperture of slit.

## 4. Discussion

The optimization of the optical parameters that determine the minimum width of the spectral lines is an important focus of research in order to obtain advancements in spectroscopic instrumentation. Spectra with low resolution present difficulties to be analyzed due to the superposition of the spectral lines of the sample. It is relevant to register that the biological applications of Raman spectroscopy in diagnosis and biochemical analysis are very auspicious, which denotes the high importance of an excellent optical system in order to promote precise measurements.

The practical implementation of Raman spectroscopy requires an integrated instrument that can provide, preferentially, real-time spectral analysis and diagnostic information to the clinician. Previously described Raman systems served as proof of principles for these concepts. Actually, using a commercially available probe to take spectra from human finger, arm, nail, tooth and tongue with acquisition times of 5 s, custom-built software was able to provide accurate classification of the different tissue types within 1 s after acquisition [9].

Although its use has been spread in the biomedical area, Raman spectroscopy has some limitations, since the diagnostic results can only be obtained when the signal-to-noise ratio (SNR) is above 10. There are problems due to sample handling on *in vivo* experiments, which cannot be accessed directly with standard, non-fiber optic coupled Raman systems.

Therefore, it is fundamental to develop an optical system that propitiates maximum intensity of peak, through elevated light power, minimizing damages to the spectral resolution in order to obtain the better signal-to-noise ratio (SNR). Thus, the previous analysis of the optimum aperture of slit for each sample is an important pre-requisite to obtain an excellent set-up aiming the improvement in the spectral quality obtained in the Raman measurements. In this way, the proposal of the present work can be considered a significant contribution in order to allow a more adequate spectral analysis of biological samples.

## 5. Conclusions

In the present work, it was verified that the reduction in the width of the slit propitiates a high resolution to the spectroscopic signal from the sample. On the other hand, the signal intensity collected in this measurement is very slow, making the spectral analysis difficult as function of the lower signal-to-noise ratio (SNR), which is resultant in these conditions. Thus, it seems to be adequate to use the higher value of resolution that still allows resolving the thinner spectral peak of the spectrum. In this way, the increase of the spectral intensity permits to obtain a higher SNR, without to damage the resolution of the spectrum.

This methodology can be considered a significant advancement in the spectral analysis in order to be employed in several clinical applications. For example, we can mention the reduction of the time for diagnosis of the calcification of coronarian tissue, which could be developed by *in vivo* analysis. This advancement will allow a more efficient employment of the diagnostic methods based on laser and spectroscopic techniques, since this kind of procedure is associated to a lower time of diagnosis of the biological tissues.

Therefore, the present work, in association with previous articles that demonstrated representative advancements in the system of data collection via new prototypes of optical fiber catheters [23,24] is a significant contribution to the biomedical sciences. In fact, these studies have furnished new procedures to biochemical analysis and pathological diagnosis to the physicists and researchers of several areas, such as the cardiovascular diseases.

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