Fluorescence Background Problem in Raman Spectroscopy: Is 1064 nm Excitation an Improvement of 785 nm?

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Abstract

The interference of background fluorescence is usually to be reckoned with in Raman analysis of organic molecules. Utilization of near-infrared excitation at 785 nm has been the standard in addressing the background fluorescence. Recently more and more vendors are introducing 1064 nm systems; however, a systematic comparison of the two wavelengths have not been carried out to determine whether 1064 nm excitation presents any significant merits in tackling the fluorescence background. To that effect, fluorescence background in Raman spectra of multiple biological samples were measured and compared using 785 nm and 1064 nm excitation. Fluorescence of Raman spectra excited by 1064 nm was over 500 times weaker than that obtained by 785 nm. Furthermore, the background was more stable in 1064 nm such that background reduction with photobleaching was minimal. In conclusion, 1064 nm presents significant merits over 785 nm in dealing with the background fluorescence.

Introduction

Auto-fluorescence is a major challenge in Raman spectroscopic analysis of organic and biological specimens [1-6]. Although fluorescence can be dealt with a variety of techniques such as the utilization of confocal configuration, photobleaching or chemical bleaching[7], the most effective way of removing or reducing fluorescence has been via the deployment of laser excitation at longer wavelengths. While longer wavelength reduced the background fluorescence burden, the Raman scattering intensity is inversely proportional to the fourth order of the excitation wavelength. Therefore, shorter excitation may be desirable to improve the sensitivity.

Photobleaching of samples by prolonged exposure of may lead to a decrease in auto fluorescence. The mechanism of photobleaching is not well understood, but in practical Raman spectroscopy applications, photobleaching is often used especially when longer wavelength excitation is unavailable. Golcuk et al. demonstrated the collection of Raman signal from bone tissue with 532 nm laser excitation after the samples subjected to 30-120 minutes photo-bleaching [8]. Sample damage and increased data acquisition time are key limitations associated with photobleaching.

Utilization of 785 nm laser has been the standard in addressing sample fluorescence, particularly in biological specimens.. A recent Raman spectroscopy study on monosodium urate (MSU) crystals isolated from joint fluids at 785 nm [5] indicated that

Raman signal can be acquired without photobleaching, however, the signal to noise ratio is improved when a brief photobleaching (1 min) is employed. Although 785 nm is largely indicated for Raman analysis of biological/organic analytes [9, 10], there are circumstances when such systems fail to acquire Raman spectra due to high fluorescence [6, 11].

Fourier-transform Raman system based on 1064 nm has existed for more than two decades; however, FT-Raman systems required long integration times (typically >30 minutes [12-14]). The emergence of more affordable InGaAs based CCD array detector now enable a burst of commercial dispersive 1064 nm Raman systems, in the recent few years. Raman studies using dispersive 1064 nm Raman systems are emerging [11, 15, 16]. At the present, there does not seem to be a comprehensive comparative analysis of 785 nm and 1064 nm in terms of their immunity to fluorescence. This knowledge is essential to the potential buyers in the market to determine whether there will be an added value in association with the choice of dispersive 1064 nm systems. In addition, the dynamics of photobleaching at 1064 nm excitation is unknown. A detailed comparison of the fluorescence recorded from the same samples set using a 785 nm and a 1064 nm Raman system is presented in this report. The reduction of fluorescence regarding to the time interval of photobleaching is also provided.

Material and methods

Three different types of biological specimens were used in this study. A slice of bovine bone, a sample of monosodiumurate crystal that was isolated from human joint fluid (as described in)[5], and a human tooth. Human tissues were collected under the approvals of Institutional Review Boards. The human tooth had a white lesion on part of the enamel surface, and dark colored spots were present within the white lesion.

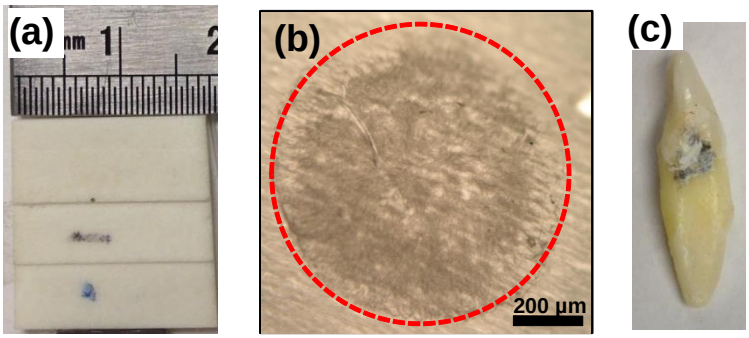


Fig. 1, Images of samples: (a) Bovine bone slice; (b) MSU crystals; (c) Human tooth with lesion.

Both 785 nm and 1064 nm Raman systems were from Wasatch Photonics Inc. The 785 nm Raman system (785L) included a 785 nm laser (Innovative Photonics Solutions, NJ) and an f/1.3 thermo-cooled spectrometer integrated with a NIR enhanced sensor (Hamamatsu S10420-1006, Bridgewater, NJ). A gelatin based volume phase holographic transmission grating (Wasatch Photonics Inc.) was used to maximize diffraction efficiency. A single lens with 25 mm focal length was used to deliver the laser light on

the sample as well as collect the Raman signal. The 1064 nm system was a fiber probe based half-commercial prototype. It included a spectrometer based on InGaAs CCD camera (Du490A-1.7, Andor, UK), and an 800 mW 1064 nm laser (Innovative Photonics Solutions, NJ). The focal distance of the lens integrated in the Raman probe is 10 mm.

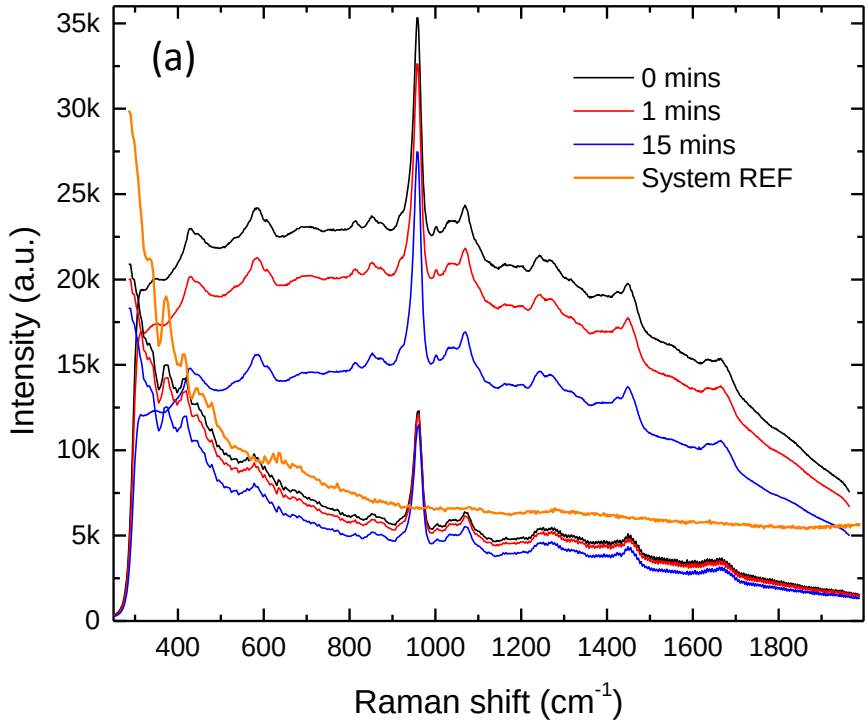
The power used for photobleaching was set at 50 mW for the 785L; the exposure time was 1 second and the spectra were averaged 10 times resulting in 10 seconds total integration. For the 1064 nm system, 500 mW laser beams was used for photobleaching, while spectra were acquired as a single scan with 10 seconds integration. The sample was placed and adjusted to the focal point of the focusing lens while the laser power was set at 5% of the photobleaching level prior to the start. The first spectrum was acquired as soon as photobleaching process started; the ensuing spectra were then recorded according to the duration of photobleaching. The noise level is determined by the standard deviation of the data points at Raman signal free region (1150 – 1200 cm^{-1}).

Results and discussion

The Raman spectra on dried bovine bone indicated that the fluorescence level is higher when it was illuminated by 785 nm laser (Fig. 2, top 3 curves) than by 1064 nm laser (Fig. 2, bottom 3 curves). The orange curve is the system response which was taken by the 1064 nm system from microscopy glass slides which are fluorescence-free. One possible source of this system response was the silica Raman background from the fiber

probe[17]; stray lights due to random scattering may also have contribution. Comparing the baseline of the bone data and the system response, it was concluded that the system response contributed largely to the baseline of the bovine bone that was recorded by the 1064 system. Taking into account the difference in laser power and the integration time, the fluorescence intensity from 785 nm system was over 500 times (in counts) more than that from the 1064 nm system before photobleaching. Higher ratio was expected if the baseline contributed from the system response is subtracted, as shown in Fig. 2b. The spectra of bovine bone after system response correction should have less fluorescence background. No studies were performed to compare the system response from different samples (which reflect excitation laser back into system); the system response in the case of bovine bone was arbitrarily estimated as 50% of that of glass slides.

Regardless the initial intensities, the fluorescence level experienced a reduction with photobleaching experiments on bovine bone for both 785 nm and 1064 nm systems. After 1 minute of photobleaching, the reduction of the two systems are 12% and 5%, respectively; their rate changed to 35% and 15%, accordingly after progressed to 15 minutes. Yang et al. reported that the high level fluorescence background introduce noise and thus affects the signal to noise ratio [5]. The noise level variations after 15 minutes photobleaching were significant (reduced 20%) with 785 nm illumination, but the variation was negligible (2%) with the 1064 nm illumination. The baseline system response from the 1064 nm Raman system did not change after 20 minutes photobleaching.


(a)
0 mins
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Intensity (a.u.)
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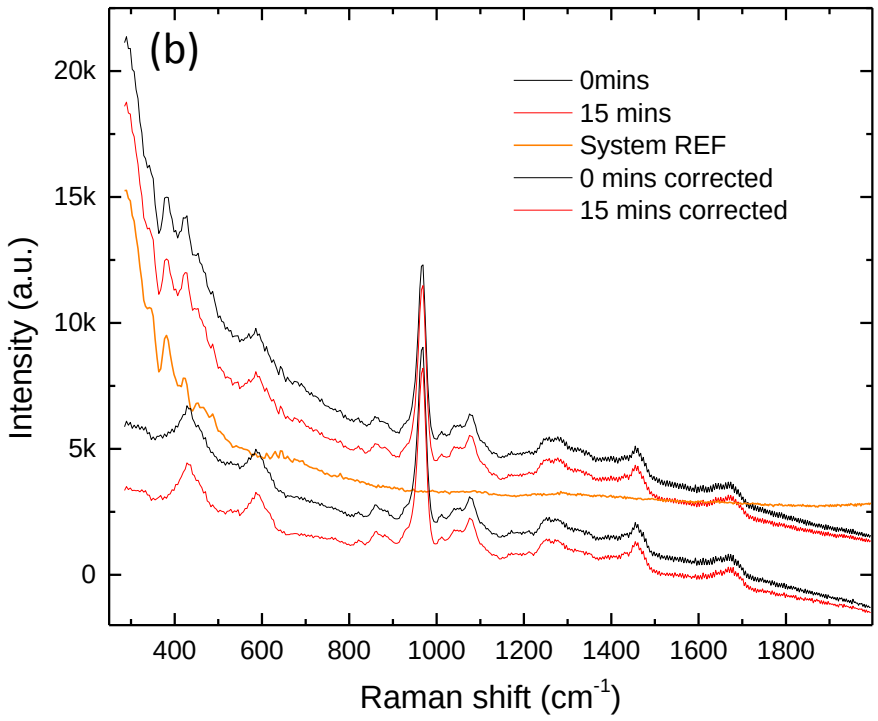
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20k
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1200
1400
1600
1800

Fig. 2. (a) Time-dependent Raman spectra of bovine bone acquired by the 785 nm (upper set) and the 1064 nm (lower set) Raman system. Black, red, and blue curve indicated 0, 1, and 15 minutes photobleaching respectively. Orange curve was the system response of the 1064 nm where a glass slide was used to acquire the baseline system response. (b) Raman spectra before and after system response correction. Accurate system response was unknown; it was arbitrarily estimated to be 50% of that in the case of glass.

A similar series of spectra from MSU crystals isolated from synovial fluid presented a faster reduction (8000 counts in 1 minutes, Fig 3) in respondse to photobleaching of 785 nm system, while the photobleaching rate in response to 1064 nm system was low (320 counts in 1 minute, Fig. 3). After 1 minute of photobleaching, the reduction of fluorescence at 785 nm and 1064 nm were 26% and 6%, respectively. The reductions at 15 minutes were 43% and 13%.

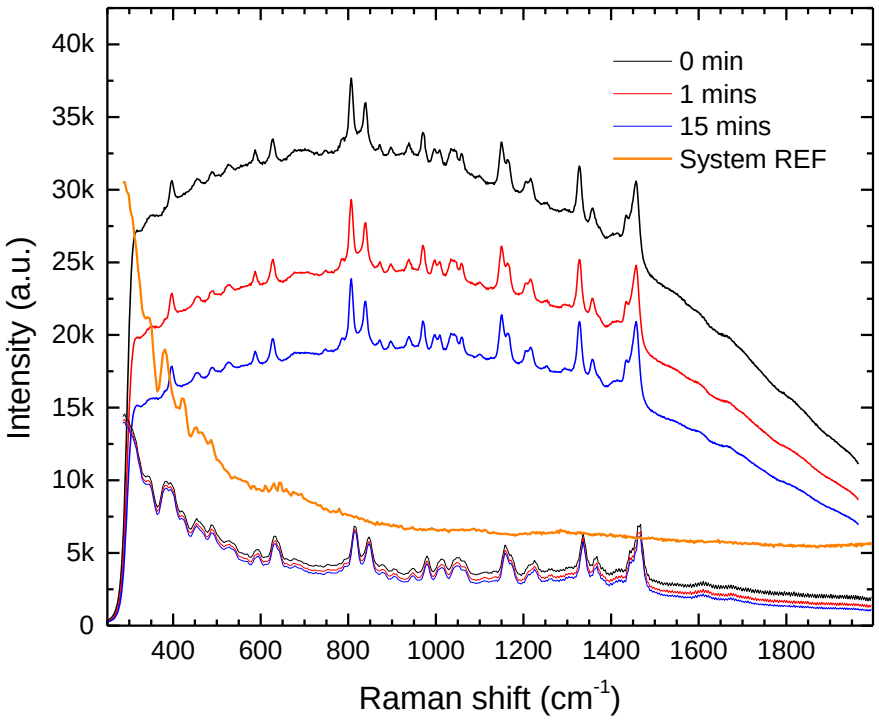

Fig. 3. Time-dependent Raman spectra of MSU taken by 785 nm (upper set) and 1064 nm (lower set) Raman system. Orange curve was the system response of the 1064 nm where a glass slides was used as the sample to acquire the baseline system response.

White lesions on human teeth normally indicated demineralized enamel and had higher concentration of organic phases than healthy enamel. In Raman spectroscopy, the lesion region showed lower mineral intensity but higher fluorescence background (data not shown). There were dark spots within the white lesion from which the 785 nm system was unable to record spectra due to intense fluorescence which saturated the system, even after 1 hour of photobleaching at 0.5 s data integration time. Raman spectra were

successfully recorded from the same intensely fluorescent spot when the 1064 nm Raman system was used (Fig. 4). The fluorescence reduction was 2%, 10% after 1 and 10 minutes photobleaching, respectively.

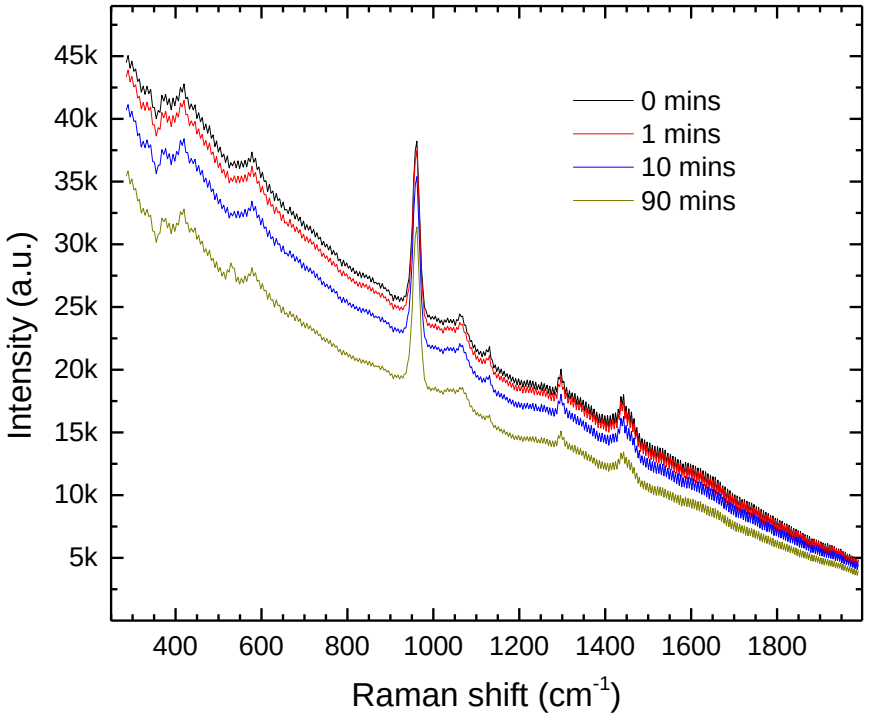

Fig. 4. Time-dependent Raman spectra of opaque spot on tooth lesion taken by 1064 nm Raman system.

Conclusion

A study comparing fluorescence background in Raman spectroscopic analysis of various biological samples at 785 nm and 1064 nm was performed. The background fluorescence was more than 500-fold lower and more stable at 1064 system. Fluorescence

from several samples was so intense to saturate 785 nm; whereas the 1064 nm enabled Raman analysis from the most fluorescent of the spots we encountered. In the overall, the users who are facing a diverse set of biological specimens may benefit more from a 1064 nm wavelength as 785 nm is not fully immune from background fluorescence in limited occasions.

Acknowledgements

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