

Studies of minerals, organic and biogenic materials through time-resolved Raman spectroscopy

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ABSTRACT

A compact remote Raman spectroscopy system was developed at NASA Langley Research center and was previously demonstrated for its ability to identify chemical composition of various rocks and minerals. In this study, the Raman sensor was utilized to perform time-resolved Raman studies of various samples such as minerals and rocks, Azalea leaves and a few fossil samples. The Raman sensor utilizes a pulsed 532 nm Nd:YAG laser as excitation source, a 4-inch telescope to collect the Raman-scattered signal from a sample several meters away, a spectrograph equipped with a holographic grating, and a gated intensified CCD (ICCD) camera system. Time resolved Raman measurements were carried out by varying the gate delay with fixed short gate width of the ICCD camera, allowing measurement of both Raman signals and fluorescence signals. Rocks and mineral samples were characterized including marble, which contain CaCO₃. Analysis of the results reveals the short ($\sim 10^{-13}$ s) lifetime of the Raman process, and shows that Raman spectra of some mineral samples contain fluorescence emission due to organic impurities. Also analyzed were a green (pristine) and a yellow (decayed) sample of Gardenia leaves. It was observed that the fluorescence signals from the green and yellow leaf samples showed stronger signals compared to the Raman lines. Moreover, it was also observed that the fluorescence of the green leaf was more intense and had a shorter lifetime than that of the yellow leaf. For the fossil samples, Raman shifted lines could not be observed due the presence of very strong short-lived fluorescence.

INTRODUCTION

Raman spectroscopy has been demonstrated to be a powerful technique for analysis of inorganic and organic minerals in any physical phase, able to determine the chemical composition and structure of a given material nondestructively and requiring no sample preparation.¹⁻⁵ A Raman spectrum results from excitation of various vibrational modes of a material. The Raman spectra of molecules and polyatomic ions consist of sharp, well-defined lines that can be used as fingerprints for unambiguous identification. With some materials, however, the same excitation also results in fluorescence emission from molecules or ions that have been excited to higher energy levels by absorption of electromagnetic radiation.⁶ This fluorescence emission can be utilized to measure trace amounts of transition metal ions and rare earth ions, to which Raman spectroscopy is generally insensitive.

A combined Raman spectroscopy and laser-induced fluorescence system previously reported by Bozlee, et al. demonstrated that laser-induced fluorescence (LIF) of rocks and minerals could be a useful technique for detecting the presence of trace cations in minerals, and provides complementary information to Raman spectroscopy.⁶ LIF can be utilized as a sensitive method for detecting organic and biological molecules.^{6,7} Fluorescence from organic and biological molecules have very short lifetime (a few ns), whereas inorganic ions have longer fluorescence lifetime (μ s to ms). Moreover, the cross section of molecular fluorescence can be several orders of magnitude greater than the Raman cross-section, making LIF easier to detect. The separation of the Raman and fluorescence spectra can be achieved by pulse gating of the detection system.

This compact remote Raman system developed at NASA Langley Research Center in collaboration with University of Hawaii was utilized to perform time-resolved Raman spectroscopy of various samples, demonstrating its ability to obtain fluorescence information from the same spectrum obtained for Raman analysis. The objective of this paper is to demonstrate the capability of the compact remote Raman system to acquire backscattering Raman and LIF spectra from calcite, green and yellow leaves, and fossil samples with a very short detector gate width (a few nanoseconds) at varying gate delay, which is required to perform time-resolved Raman analysis.

Carbonate mineral samples commercially acquired from Ward's Natural Science were analyzed. Locally acquired fresh (green) and decayed (yellow) leaves of *Gardenia* (*Gardenia Volkensii* K. Schum.) were also analyzed. Finally a few fossil samples also from Ward's Natural Science were analyzed.

EXPERIMENTAL SETUP AND PROCEDURE

The compact remote Raman spectroscopy system utilized to perform the time-resolved Raman measurement was developed at NASA Langley Research Center. The schematic diagram of the Raman system is depicted in Fig. 1. Details of the system are described elsewhere^{5, 8}, including its ability to perform remote measurements, identify various rocks and minerals, and detect water and water-bearing minerals. The Raman instrument uses as an excitation source a frequency doubled Nd:YAG pulsed laser (Big Sky Laser, Ultra CFR) to produce a 532 nm beam, having a pulse width of 8 ns and an energy of 23 mJ, with a maximum of 20 Hz pulse rate. The laser beam is made collinear to the optical axis of the telescope using two half-inch tall 45° prisms. This collinear configuration of the laser beam and the collecting telescope ensures maximum collection of the 180° backscattered radiation and maximizes the field of view of the system. The laser beam excites the samples placed on a platform about 5.6 m away from the instrument, and the excitation of the molecules in the sample results in relatively strong elastic (Rayleigh) scattering, as well as weak inelastic (Raman) scattering. The backscattered signal is collected by 4" Maksutov-Cassegrain telescope (Meade ETX-105), and then coupled to a spectrograph (Kaiser Optical Systems Inc.) by a 20x microscope objective lens. Within the spectrograph, the signal is passed through a supernotch filter to eliminate the strong Rayleigh component, the rest of the signal passed through a 100 μm slit, and a grating. An intensified CCD (Princeton Instrument) camera with 1024 x 256 pixels and 26 x 26 μm pixel size is coupled to the output of the spectrograph to view and record the resulting spectrum. The ICCD camera detects extremely weak Stokes lines diffracted by the grating.

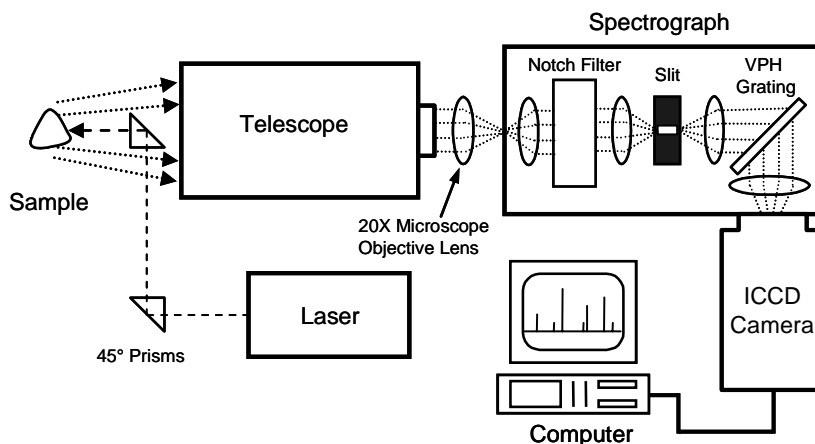


Figure 1. Schematic diagram of the remote pulsed laser Raman and LIF spectroscopy system.

Previous measurements made with this instrument were primarily aimed the Raman spectra of various samples to observe the Raman lines and bands, which were unique characteristics of each sample. In order to produce high-quality Raman spectra with very low background signal, the detector was gated in such a way that the gate can coincide with the laser pulse. Whereas low background was observed with a gate width of 2 μs , a optimum gate width of 22 ns resulted in spectra with even less background.⁸ Furthermore, it was observed from previous measurements that the Raman spectra of some samples do not only contain the Raman lines and bands, but also contain broad fluorescence signal.

In order to analyze both Raman and fluorescence spectra of the samples, a time-resolved measurement was performed using the Raman system. A timing diagram depicted in Fig. 2 shows the scheme used for detector gating to achieve time-resolved Raman spectra. The laser controller outputs a trigger signal synchronized with the laser Q-switch a few nanoseconds before the laser fires. This trigger signal is sent to the camera controller to be used as the starting point of the delay time before the camera is gated on. Time-resolved measurement is carried out by using a very short fixed gate width on the detector, then varying the gate delay to allow snapshots of different instances of scattering from the time that the laser pulse first strikes the sample, to the time Raman and fluorescence scattering occurs, up to the time the scattering ceases. A gate width of 5 ns was used and the gate delay was set from 75 ns and gradually increased by 5 ns increments up to 120 ns.

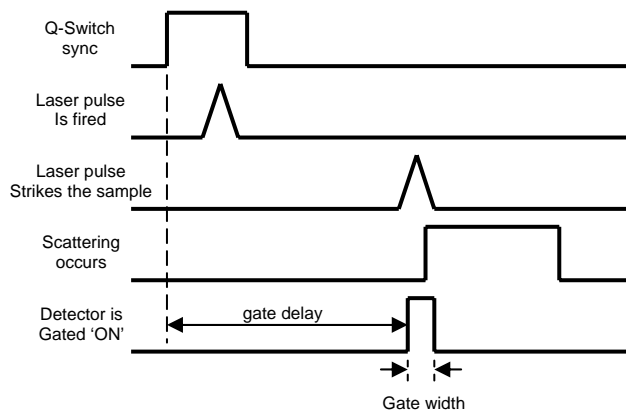


Figure 2. Timing diagram used to gate the detector for time-resolved Raman measurement. The laser Q-switch sync signal is used to trigger the laser.

RESULTS AND DISCUSSION

1. Calcite and Marble

Fig. 3 shows a set of time-resolved Raman spectra of the first calcite sample obtained with increasing gate delay starting at 80 ns (bottom spectrum) up to 115 ns (topmost spectrum) in 5 ns increment. It is observed at 80 ns gate delay, no scattering has occurred, as the laser beam has not-reached the sample. At around 90 ns, a very weak line detects at 1085 cm^{-1} corresponding to the Raman symmetric stretching mode of the carbonate molecules in the crystalline CaCO_3 . More Raman lines detect at 95 ns delay and these show the strongest intensity. At 105 ns gate delay, sharp Raman-shifted lines are detected at 156 , 282 , 711 , and 1434 cm^{-1} , which are the Raman fingerprints of CaCO_3 in calcite structure.^{9,10} The Raman signal presents within a short interval of 20 ns indicating the fast lifetime of the Raman process as the line-width of the laser is ~ 10 ns. This sample of calcite is very clear and almost transparent, showing no immediately apparent evidence of any impurities. No broad feature indicating fluorescence was observed up to 115 ns gate.

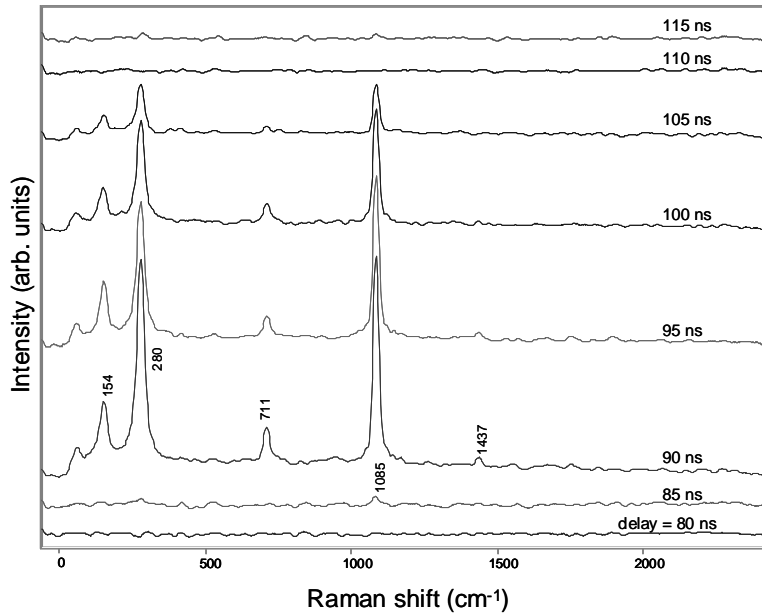


Figure 3. Time resolved Raman spectra of first calcite sample showing Raman lines taken from 85 ns to 115 ns gate delay with fixed gate width of 5 ns .

In Fig. 4, the time-resolved Raman spectra of second calcite sample (from Ward's economic minerals and rocks set) are shown. Similar to the previous calcite sample, no Raman signal was detected until a gate delay of around 90 ns. Besides the Raman shifted line at 1085 cm^{-1} , a broad spectrum centered around 500 cm^{-1} (546.6 nm) was detected due to the fluorescence in the sample. At longer gate delays, the fluorescence signal becomes larger, overlapping the relatively weaker fingerprint lines of calcite that were clearly observed in the previous calcite sample. Both the Raman and the fluorescence signals become weaker around 110 ns delay and finally disappear around 115 ns. The short lifetime of this fluorescence indicates the presence of organic molecules within the sample. This could be due to impurities contained in the material or from lipids absorbed by the material from handling the sample.

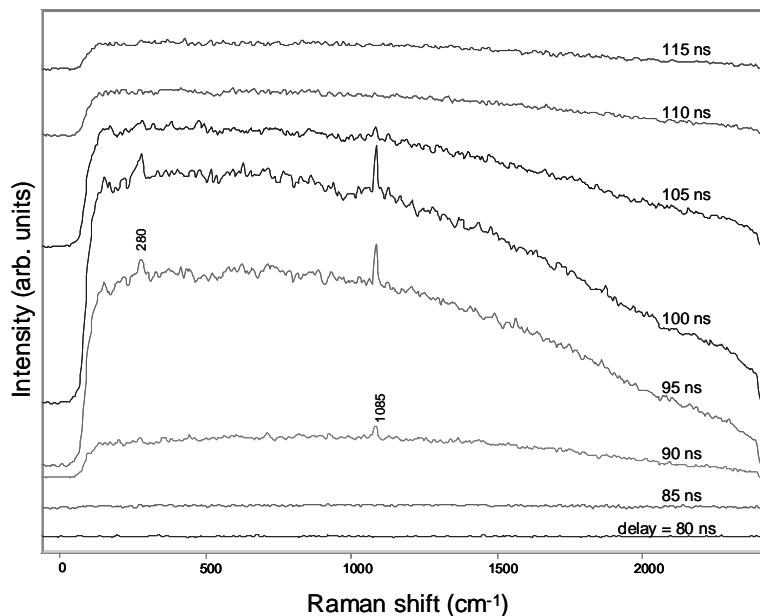


Figure 4. Time-resolved spectra of second calcite sample showing both Raman lines and fluorescence emission at gate delay from 80 ns to 115 ns with a fixed gate width of 5 ns.

In addition, a third calcite sample acquired from Chihuahua, Mexico, was tested and analyzed. Its time-resolved Raman spectra are displayed in Fig. 4. Similar to the 2 previous calcite samples, the sharp carbonate line as well as the fingerprint lines of calcite appears at 95 ns gate delay, and was observed up to 110 ns delay. It is also seen very clearly that a broad fluorescence feature centered around 1700 cm^{-1} (586.6 nm) is present, and its location is different from the position of fluorescence maxima in the previous sample, which indicates the presence of organic molecules in the sample. The impurity is caused by the short-lived fluorescence, and is different from the second calcite sample.

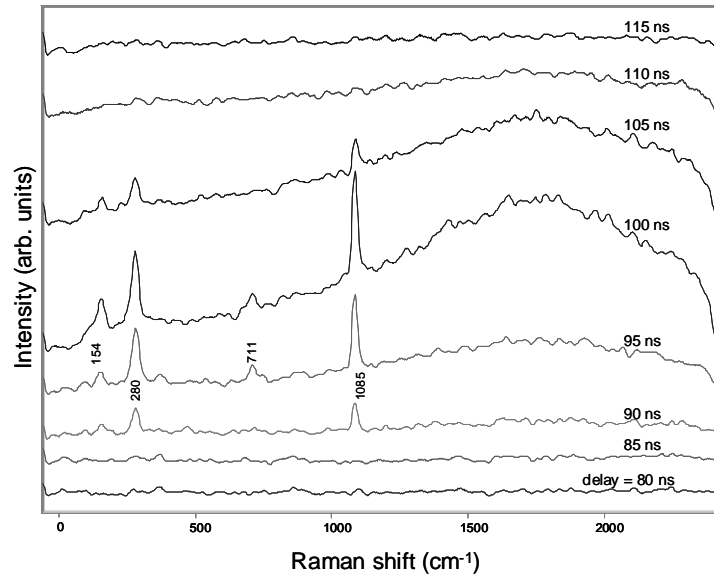


Figure 5. Third calcite sample showing Raman lines and short lifetime fluorescence, detected using a fixed gate width of 5 ns with gate delays from 80 ns to 115 ns.

A marble sample (metamorphic rock composed mainly of calcite) was tested and also analyzed with the Raman and LIF system. The time-resolved Raman spectra of the marble sample are shown in Fig. 6. The marble sample Raman spectrum contains the sharp carbonate line as well as the calcite Raman fingerprint lines. This marble, obtained from Tate, Georgia produced very sharp Raman lines with very minimal background and no fluorescence signal indicating absence of organic as well as rare-earth and transition metal ions in the sample.

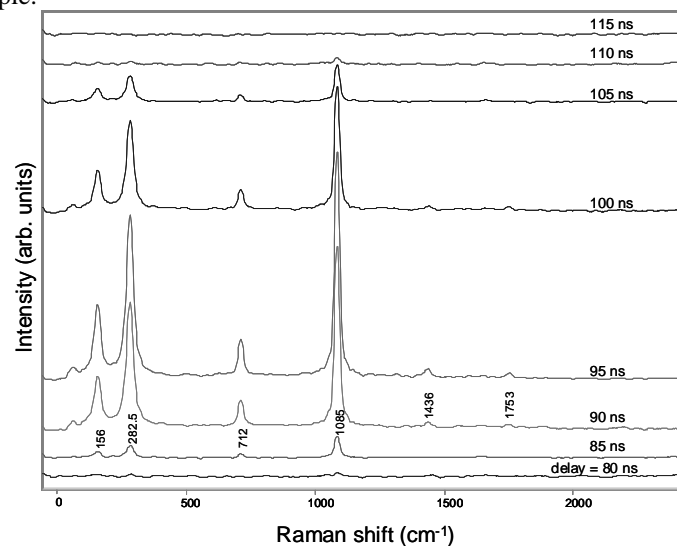


Figure 6. Time resolved Raman spectra of Marble spectra taken using gate delays from 80 ns to 115 ns with a gate width of 5 ns.

2. Leaves

Two locally obtained gardenia leaves, one pristine (green) and one decayed (yellow), were evaluated using a fixed gate width of 5 ns and a varying gate delay from 75 ns to 115 ns. The high frequency region of the time-resolved spectra of the leaves are shown in Fig. 7, where the fluorescence band due to chlorophyll-*a* centered around 4091 cm^{-1} (680.1 nm) and 3937 cm^{-1} (673.1 nm) are observed, respectively, in the spectra of green and yellow gardenia leaves. The presence of 680.1 nm fluorescence band in the spectrum of the green leaf is attributed to chlorophyll-*a* in the leaf.^{6,7,11} The intensity of the fluorescence band has much higher intensity in the green leaf compared to that in the yellow leaf and is also shifted toward lower wavelengths. This difference is due to decay of chlorophylls in the yellow leaf.^{12,13} Furthermore, the lifetime of fluorescence in the green leaf is shorter and centered at lower frequency. Fig. 8 shows the peak intensity of the fluorescence for each leaf sample with respect to the gate delay.

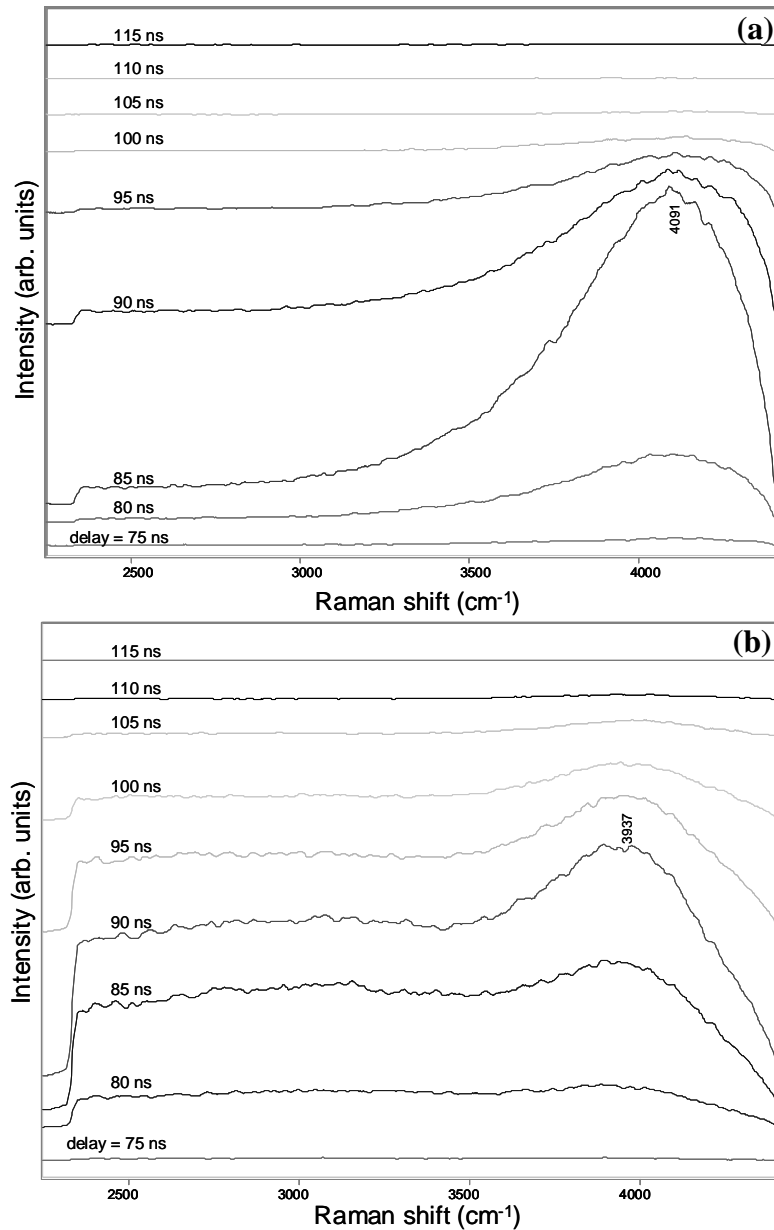


Figure 7. Time resolved Raman spectra of (a) green and (b) yellow Gardenia leaf with gate delay from 75 ns to 115 ns, with a fixed gate width of 5 ns.

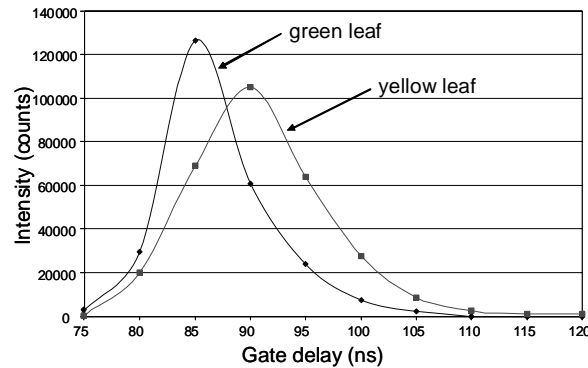


Figure 8. Intensity of the chlorophyll-a fluorescence of the green and the yellow leaf plotted against gate delay time showing the stronger intensity and shorter lifetime of the emission from the green leaf.

3. Fossils

Two fossil samples, a copal and a pelecypod both from the Miocene period, were evaluated. Their time-resolved Raman spectra are shown in Fig. 9. The Raman spectra of the three fossil samples show the presence of a broad fluorescence feature. This fluorescence signal is stronger than the Raman signal, and therefore, no Raman lines were detected. The very short lifetimes of the fluorescence features of the fossil samples indicate the presence of bio molecules from the past life in these samples.

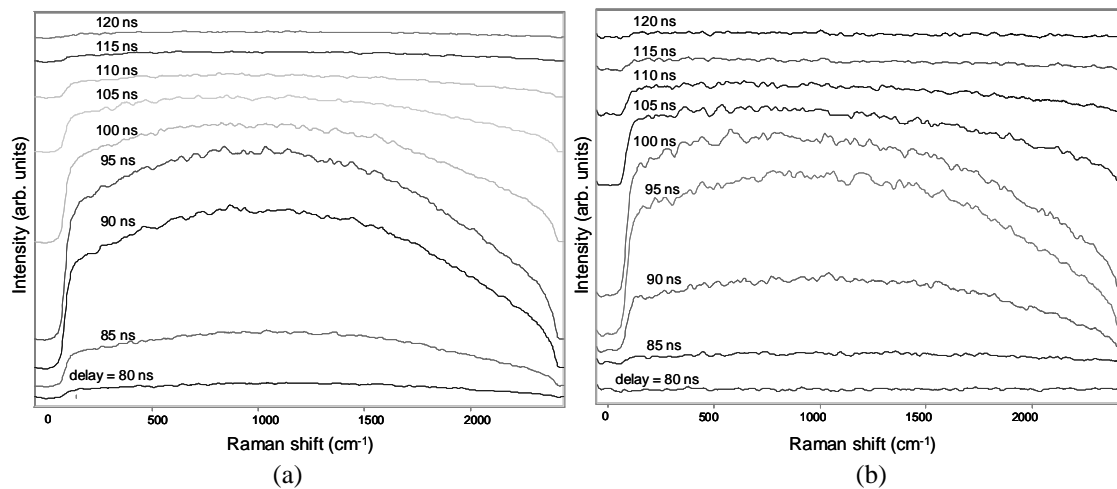


Figure 9. Time resolved Raman spectra of fossil samples, (a) copal and (b) pelecypod , from the Miocene period, showing short lifetime fluorescence.

CONCLUSION

The remote Raman system was demonstrated at 5.6 m distance for its ability to obtain Raman signal using a very short gate width (5ns), allowing time-resolved Raman measurement to determine the presence of bio-organic molecules in the material. Raman spectra of calcite and marble samples show Raman lines of carbonate ions and Raman fingerprints of CaCO_3 crystallized in calcite structure. Some of the carbonate samples produced Raman scattering, and also generated short-lived fluorescence emission indicating presence of organic molecules in the material. The time-resolved Raman spectra of Gardenia leaves revealed that intensity and lifetime of the fluorescence emission is related to the amount of chlorophyll-a and decayed products of chlorophylls presence in the leaves. The chlorophyll-a fluorescence from the green leaf at 680.1 nm has a stronger intensity and faster lifetime than that of the yellow leaf in which

chlorophylls have decayed. The fossil sample most likely contains trace amount of bio-organic molecules that exhibited short-lived fluorescence emission as expected.

REFERENCES

1. S. K. Sharma, S. M. Angel, M. Ghosh, H. W. Hubble, P. G. Lucey, "Remote pulsed Raman spectroscopy system for mineral analysis on planetary surfaces to 66 Meters," *Applied Spectroscopy*, **56**, 699-704, 2002.
2. A. K. Misra, S. K. Sharma, C. H. Chio, P. G. Lucey, B. Lienert, "Pulsed remote Raman system for daytime measurements of mineral spectra," *Spectrochim. Acta Part A*, **61**, 2281-2287, 2005.
3. J. D. Stopar, P. G. Lucey, S. K. Sharma, A. K. Misra, G. J. Taylor, H. W. Hubble, "Raman efficiencies of natural rocks and minerals: Performance of a remote Raman system for planetary exploration at a distance of 10 meters," *Spectrochim. Acta Part A*, **61**, 2315-2323, 2005.
4. S. K. Sharma, A. K. Misra, P. G. Lucey, S. M. Angel, C. P. McKay, "Remote pulsed Raman spectroscopy system of Inorganic and organic materials to a radial distance of 100 meters," *Applied Spectroscopy*, **60**, no 8, 871-876, 2006.
5. C. S. Garcia, M. N. Abedin, S. K. Sharma, A. K. Misra, S. Ismail, U. N. Singh, T. F. Refaat, H. E. Elsayed-Ali, S. P. Sandford, "Remote pulse laser Raman spectroscopy system for detecting water, ice, and hydrous minerals," *Proceedings of SPIE*, **6302**, 630215/1-630215/7, 2006.
6. B. J. Bozlee, A. K. Misra, S. K. Sharma, M. Ingram, "Remote Raman and fluorescence studies of mineral samples," *Spectrochim. Acta Part A*, **61**, 2342-2348, 2005,
7. S. K. Sharma, A. K. Misra, P. G. Lucey, "A combined Raman and fluorescence spectrometer system for detecting inorganic and biological materials," *Proceedings of SPIE*, 6409, 64090K1-64090K-9, 2006..
8. C. S. Garcia, M. N. Abedin, S. K. Sharma, A. K. Misra, S. Ismail, S. P. Sandford, H. E. Elsayed-Ali, "Remote Raman sensor system for testing of rocks and minerals," *Proceedings of SPIE*, **6302**, 630215/1-630215/7, 2007.
9. NIST Standard Reference Database Website: <http://webbook.nist.gov/chemistry>.
10. W. D. Bischoff, S. K. Sharma, F. T. McKenzie, "Carbonate ion disorder in synthetic and biogenic magnesian calcites; a Raman spectral study," *Am. Mineral.*, **70**, 581-589, 1985.
11. G. G. Matvienko, A. I. Grishin, O. V. Kharchenko, O. A. Romanovskii, "Application of laser induced fluorescence for remote sensing of vegetation," *Optical Engineering*, **45**, 156201/1-156201/5, 2006.
12. P. Q. Keegan, Leaf decay and autumn tints, *Nature* **62**, 523-524, 1900.
13. P. Folly and N. Engel, Chlorophyll *b* to Chlorophyll *a* Conversion Precedes Chlorophyll Degradation in *Hordeum vulgare* L., *J. Biol. Chem*, Vol. 274, Issue 31, 21811-21816, 1999.