Resonance Raman spectroscopy to characterize bacteria, and potential for future planetary missions

J.H. Hooijschuur, F. Ariese, Biophotonics and Medical Imaging, LaserLaB, VU University Amsterdam, Amsterdam, The Netherlands (October 2013)

Application Note

Introduction

Raman spectroscopy is a non-destructive analytical tool which needs no sample preparation and in contrast to infrared (IR) spectroscopy water is no source of interference. This technique has many applications, including the possibility for the in situ detection of biomarkers of extraterrestrial life on planetary rovers. There will be a Raman spectrometer on-board of the 2018 ExoMars mission of the European Space Agency (ESA). [1] In preparation for subsequent missions in a more distant future, we are investigating the potential advantages of more versatile Raman approaches. [2] Raman bands are usually fairly sharp and may be recorded using excitation sources in the ultra violet (UV), visible or near infrared (NIR) part of the spectrum. Therefore, high-resolution spectrometers are required with CCD detectors that are sensitive over a broad wavelength range.

Raman spectroscopy concerns the detection of inelastically scattered photons. Monochromatic light from a laser interacts inelastically with molecular bonds causing an energy transfer between the photon and the molecule. Since the photon transfers some of his energy to a vibrational mode of the molecule, the wavelength of this photon changes. Every compound has a unique Raman signature and thus this technique can be used for the identification of unknown compounds.

Figure 1a shows a Jablonski diagram of a molecule. In a Raman experiment the transfered energy of the photon corresponds with the energy of a molecular vibration (E_R), and the detected photon carries an energy E_i - E_R. This energy difference shows up as a peak in the Raman spectrum, as shown in figure 1b. In principle any (laser) wavelength could be used to record a Raman spectrum but for some molecules the sensitivity can be spectacularly enhanced by employing a wavelength that overlaps with the electronic absorption spectrum. The level of fluorescence interference will also depend on the wavelength used.

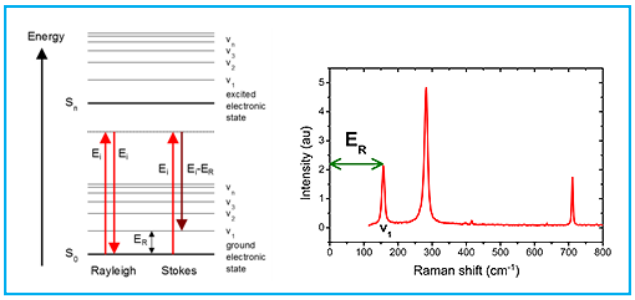

Figure 1. a) Jablonski diagram and b) a Raman spectrum where E_R is the energy of a molecular vibration

In this experiment, we tried to record a Raman spectrum of cyanobacteria. These microorganisms contain a photoactive center (chlorophyll) which can produce oxygen, just like trees. They have played a crucial role in the evolution of Life on our planet and similar species may occur or may have occurred on other planets. Chlorophylls are highly fluorescent and no Raman spectra could be recorded at an excitation wavelength of 785 nm because of the fluorescence background as shown in figure 2. For that reason, a flexible laser system can be used to optimize the signal and background.

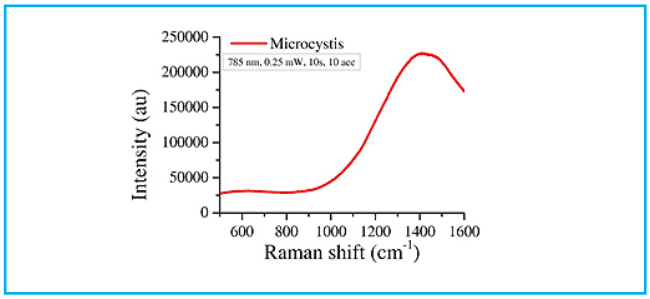

Figure 2. Raman spectra of cyanobacteria (microcystis) showing a large fluorescence background at an excitation wavelength of 785 nm.

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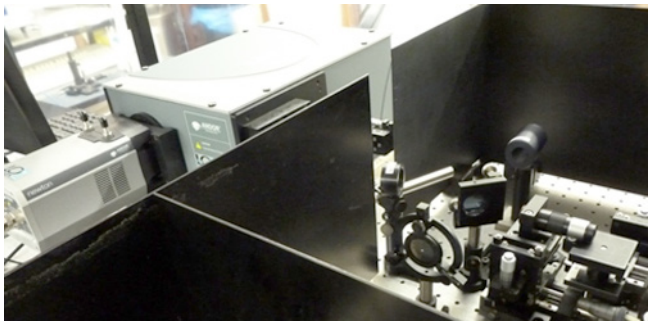
Experimental Setup



Our experimental setup consists of a 532 nm, frequency doubled Nd:YVO laser (Coherent Paladin Advanced 532-20000, Santa Clara, CA, USA) operated at 20 W and pumps an OPO laser (APE Levante Emerald, Berlin, Germany) with tunable wavelength range for the signal from 690-990 nm and an idler range from 1150-2300 nm. Both signal and idler can be frequency doubled/tripled (APE HarmoniXX); in this case the signal output was tuned to 880 nm and frequency doubled in order to obtain 440 nm excitation. The Raman signal is collected in backscatter mode through a dielectric stack long-pass filter (Semrock 450 AELP, Lake Forest, IL, USA) and focused at a spectrograph (Andor Shamrock SR-303i-A, Belfast, UK) and detected with a CCD detector (Andor Newton DU920P-BR-DD) cooled to -60 °C. Spectra are acquired in full vertical binning mode.

Results

These preliminary results are obtained at an excitation wavelength of 440 nm as shown in figure 3. Since the fluorescence of the cyanobacteria is at a different spectral region than the Raman signals, there is no interference between those signals and thus the spectrum does not suffer from a large fluorescent background. Although these measurements are done in an aqueous environment, one should be careful when using the relatively high laser power of 57 mW since it may cause photodegradation of the cells.

The Raman spectrum of the cyanobacteria shows a clear signature of carotenoids, represented by the three characteristic vibrational peaks at 990, 1140 and 1510 cm^{-1}. Future research will focus on the detection of such microorganisms in and on minerals.

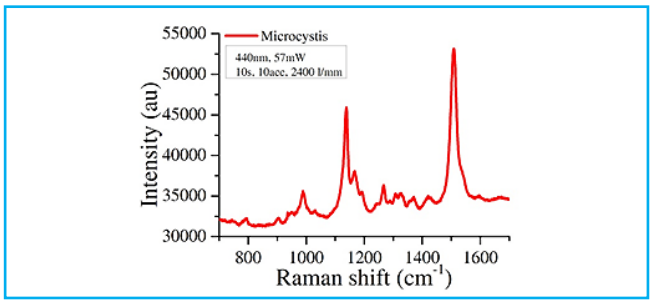


Figure 3. Raman spectra of a strain of cyanobacteria (Microcystis) recorded at an excitation wavelength of 440 nm.

Outlook

Raman spectroscopy is a powerful and non-invasive analytical method for the molecular identification of compounds. Since many different excitation wavelengths may be required for an optimal sensitivity and selectivity, CCD detectors with a low dark current and with a high quantum efficiency over a broad wavelength range are necessary. In this project, Raman spectroscopic methods will be optimized for the detection of biomarkers for future planetary missions.

References

[1] H.G.M. Edwards, I.B. Hutchinson, R. Ingley. Raman spectroscopy and the search for life signatures in the ExoMars Mission. Int. J. Astrobiol. 2012, 11, 269.

[2] J.H. Hooijschuur, I.E. Iping Petterson, G.R. Davies, C. Gooijer, F. Ariese. Time Resolved Raman Spectroscopy for depth analysis of multi-layered mineral samples", J. Raman Spectr. 2013, in press.

Contact

Dr. Freek Ariese
Associate Professor Biophotonics and Medical Imaging
LaserLaB
Faculty of Sciences
VU University
Amsterdam
The Netherlands

Phone: +31 20 59 87524
E-Mail: f.ariese@vu.nl
Web: www.nat.vu.nl/en/research/biophotonics-and-medical-imaging/index.asp