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UV Raman spectroscopy—A technique for biological and mineralogical *in situ* planetary studies

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Abstract

We report on the great advantages of using deep UV Raman system for *in situ* planetary applications. Among them are to be mentioned: (I) higher scattering efficiency compared to VIS–IR Raman excitation wavelengths, (II) electronic resonance effects which increase the intrinsically weak Raman signal thus improving the S/N ratio of the detected Raman signals and (III) spectral separation of Raman and fluorescence signals.

All these advantages are making UV Raman a valuable technique for *in situ* planetary applications. Mineral as well as biological samples were analyzed using Raman deep UV excitation and the results are presented. For the mineral samples a comparison with excitation in the NIR–VIS spectral regions is made. The impact of fluorescence on Raman data acquisition at different laser excitation wavelengths is assessed. Making use of the resonance effects, spectra of microorganisms were recorded with a high S/N ratio, allowing afterwards a very precise identification and classification (to the strain level) of the measured samples.

Keywords: UV Raman spectroscopy; Meteroites; Microorganism

1. Introduction

Raman spectroscopy [1] is a powerful method used to determine the complete chemical composition of unprepared surfaces. This method is equally well suited and applicable for the detection of minerals, organic substances, and water. This qualifies Raman spectroscopy as a valid technique for space applications. A Raman instrument can contribute to resolving various questions in the field of planetary research [2–8], e.g., the search for signs of extinct and/or extant life on Mars as well as to identify hazards for future human missions. The search for extant life needs to look for traces of sugars, phospholipids, amino acids, nucleotides (ATP/ADP). The search for extinct life, on the other hand, requires looking for organic residuals of biological origin like fossils or related geochemical and mineralogical bio-signatures. The envisaged future planetary missions require space-born instruments, which are highly miniaturized and which require as little power as possible. Space-born Raman spectrometers fulfilling these characteristics have been developed in the past years [9–13] for future Mars missions.

Raman scattering is a very inefficient process with roughly one in 10^7 scattered photons carrying the needed information. The Raman signal yield and the gained information can be maximized by carefully choosing the excitation laser wavelength in a normal Raman experiment. Raman scattering is only one of several other physical processes that might take place when light interacts with matter. Some of these processes compete with the Raman process (e.g., absorption) or/and are interfering with the detection of the weak Raman signal (e.g., fluorescence). To avoid the problem of fluorescence, two approaches are normally used. One is to lower the energy of the incoming photon such that the excitation of the molecule in an electronic state does not take place. Therefore the wavelengths of the laser used for excitation lie in the NIR region of the spectrum (from 785 nm up to 1064 nm). With this approach for most of the samples

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(especially the biological samples) the fluorescence excitation is avoided. Avoiding fluorescence in this way for the minerals does not prove to be very efficient since in minerals there is always a certain amount of rare-earth elements impurities which do have the excited electronic levels at relatively low energies.

The second approach used for minimizing the interference of fluorescence with the Raman signal is to use excitation wavelengths in the deep UV region. At these wavelengths the fluorescence is excited but no fluorescence interference exists when excitation is at wavelengths below about 250 nm [14]. A typical Raman spectral range of 4000 cm^{-1} occurs in less than 30 nm above the excitation wavelength at 250 nm. This provides complete spectral separation of Raman and fluorescence emission bands resulting in high signal to noise measurements.

In addition to having spectrally well-separated Raman and fluorescence signals, if the Raman excitation occurs within an electronic resonance band of a material then the scattering cross-section can be highly improved. Diamond, nitrites and nitrates, and many other organic and inorganic materials have strong absorption bands in the deep UV and exhibit resonance enhancement of Raman bands when excited in the deep UV [14]. This resonance effect gives extraordinary results when biological samples are to be investigated [14].

Although the Raman spectrum acts like a clear fingerprint for mineral samples and simple organics, for bacterial identification the Raman spectral fingerprint is 'blurred' by the overlapping of Raman signals generated by all bacterial constituents. Conventional bacterial identification methods currently used are based on morphological evaluation of the microorganisms and their ability to grow in various media under different conditions [15]. Depending on the type of bacteria, the identification process may take at least one day but generally much longer [16,17]. Other analytical methods such as mass spectroscopy, polymerase chain reaction (PCR), flow cytometry, or fluorescence spectroscopy were developed, which allow for a fast and reliable identification [15,16] but their application for remote based instruments (e.g., *in situ* planetary science) is not readily possible because of the miniaturization of equipment such a deployment requires.

Vibrational spectroscopy of biological samples provides information about the chemical composition of all cell components. Naumann et al. [18–21] showed that IR and Raman spectroscopy can be used to classify bacteria and yeasts. Due to the high spatial resolution of approximately 1 μm micro-Raman spectroscopy can be used both on bulk samples [22,23] and on single bacterial cells [24,25]. For eukaryotic cells like yeast cells line scans over the cells are necessary in order to overcome the spatial heterogeneity of the cells [23,26].

In order to enhance the Raman signals a special technique, the so-called surface enhanced Raman spectroscopy (SERS), with various different SERS-substrates or SERS microchips in combination with antibodies is also used for bacterial investigation [27–30].

A different method of enhancing Raman signals is resonance Raman spectroscopy. Using UV excitation direct investigation of macromolecules such as DNA or proteins becomes possible [31–35]. This allows for the measurement of microorganisms with high reproducibility [36,37]. The first attempts of a UV-resonance Raman spectroscopic identification were performed on bacteria on a genera level [32] and of the bacillus group [38].

In addition, when comparing the available Raman signal for both cases of NIR and UV excitation an increase of approximately two orders of magnitude in the Raman scattered photons can be obtained by moving from NIR (at 785 nm) to the UV spectral region (248 nm). The Raman cross-section itself is dependent on the excitation wavelength to the inverse fourth power resulting in higher Raman intensity with shorter wavelength laser excitation. The size of the sampling spot for micro-Raman experiments is proportional to the wavelength of the laser beam. A better spatial resolution for Raman mapping experiments is achieved when the excitation laser has a shorter wavelength.

All of these advantages make deep UV Raman spectroscopy a valuable technique for planetary *in situ* applications. The technical readiness needed for implementing this approach into a space-qualified instrument is currently under investigation and the results are to be published. The MIRAS 2 project run by the Institute of Chemical-Physics at the University of Jena and Kayser-Threde GmbH in Munich and financed by the German Space Agency is analyzing a possible instrument design for a deep UV Raman spectrometer.

2. Materials and methods

The capabilities of deep UV Raman excitation were investigated for two different sample systems—mineral samples (MARS meteorites) and biological systems (microorganisms). For the mineral samples comparisons between deep UV Raman excitation and excitation at other laser wavelengths were made (excitation wavelengths used were 244, 257, 532, 633 and 830 nm). The microorganisms were investigated with only the deep UV excitation using the Raman resonance effect for recording spectra with a good S/N ratio. A good S/N ratio was needed for a reliable automatic identification and classification of the microorganisms using multivariate analysis.

2.1. Spectroscopic instrumentation

UV (for mineral samples) and UV-resonance (for biological samples) Raman spectra were collected using a micro-Raman setup (HR800, Horiba/Jobin-Yvon) with a focal length of 800 mm, a 40× anti-reflection coated objective (LMU UVB) with a numerical aperture of 0.5 and a 2400 lines/mm grating. The entrance slit was 150 μm wide. Excitation wavelength of a frequency doubled line of an argon-ion laser (Innova 300, FReD) at 244 nm was used. Raman-scattered light was detected by a nitrogen-cooled CCD-camera with an accumulation time set to 120 s. Samples were prepared as dried films on silica plates according to the procedure described by Naumann et al. [19,39] and rotated at 6 rpm, moving it in x, y-direction after each rotation. For the x/y-scans the meteorites were moved relative to the fixed laser spot with help of a motorized stage.