The evaluation of time-resolved Raman spectroscopy for the suppression of background fluorescence from space-relevant samples

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Abstract
One of the primary goals in space research is the search for signs of extant or extinct extraterrestrial life, and Raman spectroscopy can play a role in this field. Raman spectrometers are planned for future missions to Mars and possibly the Moon to identify the mineralogical surface composition and potentially existing organic compounds (especially on Mars). However, a major challenge in Raman spectroscopy, especially in the visible range, is the strong fluorescence background. Time-resolved Raman spectroscopy (TRRS) can provide selective detection of Raman signals over the generally longer living fluorescence. This study investigates the potential of a TRRS system, using 3-ps, 440-nm laser pulses and time-gated detection with an intensified charge-coupled device (CCD) camera. Test samples were the lichen Xanthoria elegans as an extraterrestrial life analogue, and a lunar regolith analogue material (LRS) as a planetary surface analogue. The TRRS technique is evaluated by comparing gated to nongated Raman spectroscopy using different detectors but with otherwise the same instrument and identical measurement conditions. The gated spectra of X. elegans showed significant signal-to-noise ratio (SNR) improvements compared to the nongated spectra. The visible Raman lines could be assigned to the photoprotective pigment parietin. For the LRS sample, measurement spots with a good SNR in the nongated spectrum were not significantly improved by measuring in gated mode. However, spots dominated by fluorescence showed significant improvement in gated mode because of fluorescence suppression. Minerals such as plagioclase, diopside, olivine, apatite, and a carbonate mineral were detected. In most cases, TRRS provided better results compared to nongated measurements, demonstrating the suitability for future space-exploration missions.

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1 | INTRODUCTION

Important areas of interest in space research are the search for traces of extraterrestrial life and the study of extraterrestrial surface compositions. As part of these investigations, Raman spectroscopy will be included in future extraterrestrial missions such as ExoMars,[1] Mars 2020,[2] and possibly the European Lunar Lander.[3] Raman spectroscopy is an established method to investigate the chemical composition of materials. Because it does not require sample preparation and is nondestructive, it does not interfere with any other measurements one might want to perform on the same sample. A Raman setup has potential to detect signs of extraterrestrial life and to determine the mineralogical surface composition of an extraterrestrial body. However, a major problem for conventional (continuous excitation —nongated) Raman spectroscopy is interference from fluorescence of either organic or mineralogical origin. The main aim of this work is to investigate the capabilities of time-resolved (gated) Raman spectroscopy for the analysis of space-relevant, fluorescent samples. For this purpose, two analogue samples were chosen: lunar regolith analogue material (LRS) for mineralogical material and the extremophile Xanthoria elegans for biological material.

1.1 | Moon

The lunar surface is characterized by numerous impact craters, which cover the highlands as well as the lowlands (maria), whereby the lowlands are relatively smooth. The diameter of these craters varies from <1 μm to about 1,000 km.[4] Both terrains are easily distinguished visually, because the highlands seem bright and the maria seem dark. Regarding the mineralogy, the highlands seem to be mostly composed of feldspar, whereas the lowlands have a high abundance of pyroxene.[5] Furthermore, the lunar surface is covered by a fine-grained layer of regolith caused by continuous impact events (for at least 3–4 billion years) of meteorites and charged solar and cosmic particles.[4] The regolith particle size is <1 cm, and the estimated thickness of this regolith layer is 4–5 m (maria) and 10–15 m (highlands) on average. During the first moon missions in the late 1950s, the first lunar soil sample was analyzed by Surveyor 5. It detected oxygen (the most abundant element), silicon, aluminum, magnesium, carbon, sodium, and other elements, indicating a silicate rock.[6] With Apollo 11, the first sample-return mission was successfully completed. Further missions of the Apollo program (Apollo 12, 14, 15, 16, and 17) and the Luna program (Luna 16, 20, and 24) followed. Papike et al. published[7] a detailed summary of various studies that analyzed the chemical composition of these samples. The most abundant minerals on the Moon are the silicate minerals: plagioclase feldspar (Ca,Na)(Al,Si)4O8, potassium feldspar KAlSi3O8, pyroxene (Ca,Fe,Mg)2Si2O6, and olivine (Mg,Fe)2SiO4. Silicate minerals SiO2 such as quartz, cristobalite, and tridymite occur in large quantities on Earth but are rare on the Moon. The second most common group are the oxide minerals: ilmenite (Fe,Mg)TiO3, spinel [(Fe,Mg)(Cr,Al,Fe,Ti)2O4], and armalcolite (Fe,Mg)Ti2O5. Smaller amounts of rutile TiO2, baddeleyite ZrO2, and zirconolite [(Ca,Fe)(Zr,REE)(Ti,Nb)2O7] are also present. Metallic iron Fe and the sulfide minerals, for example, troilite FeS, are less abundant. Phosphate minerals, for example, apatite Ca5(PO4)3(OH,F,Cl) and whitlockite Ca3(PO4)2, are rarely found. Water containing minerals such as clays, micas, and amphiboles are completely absent on the Moon. Many of the above-mentioned components are also included in the lunar regolith analogue material (LRS) used in this study (see below).

1.2 | Extremophiles

Extremophiles such as lichens (e.g., X. elegans) and cyanobacteria are well-known for their ability to survive extreme environmental conditions such as low humidity and temperature,[6–10] katabatic winds,[8] and elevated ionizing UVB and UVC radiation[8,9,11,12] caused by stratospheric ozone depletion in spring (ozone hole).[13] Regions with such extreme environmental conditions can, for instance, be found in the Antarctic, and these are widely accepted as Martian analogue habitats.[8,9,14–18] Some organisms, among them different lichens or cyanobacteria, developed various survival strategies including the synthesis of biomolecular protectants. Epilithic lichens form an upper layer that is exposed to solar radiation and contains photoprotective compounds. These pigments protect the organism from photo inhibition and UV damage[8,11,17,19–24] according to two mechanisms:

1. absorption of UV radiation by pigments such as parietin in X. elegans,[8,11,25–27] which prevents harmful radiation from damaging other important molecules and
2. quenching of free radicals by, for instance, carotenoids,\textsuperscript{[11,15,17,24]} which prevents the formation of toxic singlet oxygen.\textsuperscript{[8,23]}

Another protection strategy is the colonization of “shaded areas” (endolithic lichens), for example, under the surface or inside rocks.\textsuperscript{[8,28]} This helps to shield the organisms from katabatic winds and desiccation, as well as UV radiation. Because of their remarkable survivability, lichens, cyanobacteria, and/or their components are recognized as space-relevant candidate biomarkers for potential extant or extinct extraterrestrial life in astrobiological research.\textsuperscript{[12,21,22,29–38]}

For instance, Meeßen et al. investigated the morphology and anatomy of five lichen species (\textit{Circinaria gyrosa}, \textit{Rhizocarpon geographicum}, \textit{X. elegans}, \textit{Buella frigida}, and \textit{Pleopsidium chlorophanum}).\textsuperscript{[21]} In another study, a diverse set of secondary lichen compounds (e.g., parietin, emodin, melanin, norstictic acid, carotenoids, etc.) responsible for the avoidance of photo- and UV damage\textsuperscript{[22]} was analyzed. De la Torre et al. and Sancho et al. examined the survivability of a set of lichens\textsuperscript{[29,56]} (\textit{R. geographicum} and \textit{X. elegans}) under exposure to space vacuum and radiation for 10 and 16 days, respectively, onboard the BIOPAN-5 facility of the European Space Agency. Both lichens showed a strong resistance against harsh space conditions. The influence of space and Mars-like conditions on the stability of biomolecules (chlorophyll \textit{a}, chlorophyll \textit{b}, melanin, carotene, parietin, naringenin, chitin, and murein) and the survivability of extremophiles (\textit{Circinaria gyrosa}, \textit{Buella frigida}, \textit{Cryomyces antarcticus}, \textit{Chroococcidiopsis sp.}, etc.) is and will be examined in the Biology and Mars Experiment (BIOMEX) project.\textsuperscript{[30]} This also includes the effects of planetary analogue material (Mars and Moon) on these samples. Within the framework of that project, a mixture of Mars analogue material and the carotenoid deinoxanthin (from \textit{Deinococcus radiodurans}) was exposed to solar radiation for 469 days in low Earth orbit. Subsequently, Leuko et al.\textsuperscript{[35]} investigated the degree of degradation of deinoxanthin. In another study, de Vera et al.\textsuperscript{[12,31]} found evidence of high resistance to simulated space conditions (UV radiation and space vacuum) for the lichens \textit{Fulgensia bracteata} and \textit{X. elegans} and their isolated photobiont. In ground-based simulation experiments of space and/or Mars-like conditions, the mixture of a lichen (\textit{B. frigida}) with Mars analogue material and a cyanobacterium (\textit{Chroococcidiopsis}) with Mars and Moon analogue material were studied by Meeßen et al.\textsuperscript{[34]} and Baqué et al.,\textsuperscript{[37,38]} respectively.

The potential of Raman spectroscopy as an appropriate technique for future planetary missions and as a powerful tool for the identification of organic biomarkers was confirmed in various studies,\textsuperscript{[9,14–16,32,39,40]} of which several deal with the Raman spectroscopic examination of \textit{X. elegans} and its individual components. However, biological samples often exhibit strong fluorescence when excited with visible radiation. In conventional Raman spectroscopy, this often means that the relatively weak Raman lines are buried in the shot noise of the background. To limit the level of fluorescence and thereby improve the quality of the spectra, most Raman spectroscopic analyses of \textit{X. elegans} or key biomolecules such as the highly fluorescent pigment parietin used an excitation wavelength of 1,064 nm.\textsuperscript{[17,25,26,41,42]} However, for mineralogical identification, shorter wavelengths are more suitable.\textsuperscript{[26]} The Raman intensity is inversely proportional to the fourth order of the excitation wavelength, which means that excitation at shorter wavelengths leads to higher Raman intensities. Additionally, shorter wavelengths can result in extra sensitivity and selectivity for target molecules through resonance enhancement of the Raman signal.

### 1.3 Fluorescence and time-resolved Raman spectroscopy

Various strategies to reduce the interference from fluorescence have been reviewed recently by Wei et al.\textsuperscript{[43]} When exciting in the deep UV range, the resulting fluorescence of most organic compounds would not interfere with the Raman spectrum,\textsuperscript{[44,45]} but for some samples, the risk of photochemical damage and the limited penetration depth could be a problem. With visible excitation, chemometric subtraction of the fluorescent background may help to some extent but cannot remove the associated shot noise. One promising approach makes use of the time difference between the photons due to Raman scattering (instantaneous) and the fluorescence photons that are emitted over the course of nanoseconds to microseconds, depending on the lifetime of the excited state.\textsuperscript{[46,47]} In time-resolved Raman spectroscopy (TRRS), a gated detector is used, synchronized with a pulsed laser excitation source. The pulse duration should be as short as possible for maximum temporal discrimination, but too short (femtosecond) pulses would result in significant spectral broadening due to the Heisenberg uncertainty principle. Pulses of a few picoseconds are a good compromise. Time-gated detection can be achieved using either a laser-driven Kerr gate in front of the CCD (charge-coupled device) detector,\textsuperscript{[48]} a streak camera,\textsuperscript{[49]} a complementary metal-oxide semiconductor single-photon avalanche diode (CMOS-SPAD) detector,\textsuperscript{[50]} or an intensified CCD camera.\textsuperscript{[51,52]} These options differ in terms of temporal resolution, maximum repetition rate, and instrumental complexity.

In this study, we tested the potential of TRRS with an intensified CCD (ICCD) camera to identify the chemical composition of two space-relevant samples. We...
investigated one biological sample, lichen *X. elegans* as an analogue for extraterrestrial life, and one mineralogical sample, lunar regolith analogue material (LRS) as an analogue for the surface of an extraterrestrial body—with a single excitation wavelength of 440 nm. The resulting spectra were compared with those obtained with a nongated camera, using otherwise the same instrument setup. The overall aim of this work was to evaluate the potential of TRRS in the context of future planetary missions.

2 | EXPERIMENTAL SECTION

2.1 | Samples

Two space-relevant samples—one biological and one mineralogical sample—were chosen to evaluate TRRS compared to conventional (nongated) Raman spectroscopy for space exploration.

2.2 | *Xanthoria elegans*

A lichen is a symbiotic association of a heterotrophic mycobiont (fungus: in this case *Lecanoromycetes*) and a photoautotrophic photobiont (in this case a green alga: *Trebouxiophyceae*).[22] *X. elegans* has a broad global distribution and can be found mainly on rocks of volcanic origin, silicates, and limestone rocks.[53] A special feature is its natural growth at various locations under extreme environmental conditions such as the Himalaya range[53] and the dry and cold Antarctic, for example, Victoria Land and Berry Hill on James Ross Island.[53] The investigated sample in this study was provided by the Institute of Botany of the Heinrich Heine University Düsseldorf, Germany. Originally, it was collected from the Col du Sanetsch in the Valais region of Switzerland (2,140 m).[21,22]

The schematic representation in Figure 1 shows the general structure of a lichen. A detailed description of the morphology and anatomy of *X. elegans* is given in Meeßen et al.[21] This lichen is characterized by a bright yellow-orange thallus, caused by various pigmented components, for example, anthraquinones such as parietin and emodin in the upper cortex.[21,22] The presence of these components has also previously been identified through Raman spectroscopic investigations of different parts of *X. elegans*.[8,25] Below the upper cortex follows the algal layer that consists of chlorophyll *a* and *b*, carotenes (e.g., β-carotene), and a diverse set of xanthophylls (e.g., neoxanthin, lutein, violaxanthin, antheraxanthin, and zeaxanthin). This is followed by the medulla layer of loosely arranged hyphae and the lower cortex, which like the upper cortex consists of fungal filaments. Because of the limited penetration depth at 440 nm and due to resonance effects, most of the Raman signal is expected to originate from the upper cortex.

2.3 | Lunar regolith analogue material (LRS)

For this study, a lunar regolith analogue material was produced by mixing various terrestrial silicate minerals, an oxide mineral, a phosphate mineral, volcanic slag, and iron (Table 1). It was provided by the Museum für Naturkunde, Leibniz-Institut für Evolutions- und Biodiversitätsforschung in Berlin, Germany.

| Components | Chemical formula | wt.% |
|------------|------------------|------|
| Plagioclase | (Ba, Ca, Na, K, NH₄)(Al, B, Si)₂O₈ | 66.7 |
| Volcanic slag | — | 9.5 |
| Diopside | CaMgSi₂O₆ | 8.9 |
| Hypersthen | (Fe, Mg)₂[Si₂O₆] | 5.7 |
| Olivine | Mg₁.₅Fe²⁺Fe³⁺₂[SiO₄] | 5.7 |
| Iron | — | 1.3 |
| Apatite | (Ca, Ba, Pb, Sr, etc.)₆(PO₄, CO₃)₁₂(F, Cl, OH) | 1.1 |
| Ilmenite | Fe²⁺²⁺TiO₃ | 1.1 |

*Note.* In this case, the volcanic slag is volcanic glass composed of basalt.
The individual components of this mixture were chosen based on the knowledge of the lunar surface composition. The grain size was less than 1 mm, and the powder was pressed into pellets for Raman spectroscopic investigation (Figure 2).

2.4 | Setup

Figure 3 depicts the experimental time-resolved Raman setup. It consisted of a 532 nm, frequency-doubled Nd:YVO4 laser (Coherent Verdi-V18, Santa Clara, CA, USA) that pumped a Ti:sapphire laser (Coherent Mira 900P), which was tunable from 700 to 950 nm and set to 880 nm. It produced 3-ps pulses at a repetition rate of 76 MHz (13.2 ns pulse interval). For this study, the 880 nm light was frequency doubled, yielding an excitation wavelength of 440 nm. A Pellin–Broca prism was applied to suppress traces of other wavelengths. Afterwards, the laser beam was directed through a tiny prism and onto the sample. When necessary, a gray filter was inserted into the beam path to reduce the laser power. The laser spot size on the sample was about 120 μm. After collection and collimation (using lens L2) of the light coming from the sample, it passed through a dielectric long-pass filter Semrock 450 AELP (Semrock Inc., Lake Forest, IL, USA) to block the laser wavelength. Another lens (L3) focused the Raman scattered light and the emitted fluorescence on the 100 μm entrance slit of a spectrograph (SpectraPro, Acton, MA, USA) with a 2,400 l/mm grating. Both continuous detection and time-gated detection were performed. Using a flip mirror, it was possible to switch between a nongated CCD camera (model DV420-O, Andor Technology, Belfast, UK) and an intensified CCD camera (LaVision Picostar HR, LaVision GmbH, Göttingen, Germany). The ICCD was triggered using a photodiode and an electronic delay line that could be automatically incremented with a minimum step size of 25 ps. The cameras were operated at temperatures of 228 K (nongated) and 262 K (gated). In this study, a gate width of approximately 250 ps was used. These settings were based on a publication by Efremov et al.[52]

FIGURE 2  Left: Pellets of lunar regolith analogue material (LRS). Right: Microscope image of the surface of the LRS pellet. The scale bar is 200 μm

FIGURE 3  Schematic representation of the time-resolved Raman setup
2.5 | Data analysis

The measurements were performed in two steps. First, the optimal parameters for time-resolved Raman measurements of the specific sample were determined. After each time-gated measurement, a nongated Raman spectrum was recorded from the same spot, using the same excitation conditions and total measurement time per spectrum. Outliers such as cosmic rays and hot pixels were removed or suppressed. Then the gated and nongated measurements were compared by calculating the signal-to-background ratio (SBR) and the signal-to-noise ratio (SNR). The SBR is defined as the ratio of peak height of the Raman line to the height of the fluorescence background at the same position as the Raman line. The SNR is the ratio of the peak height of the Raman line to the noise. The noise was determined by taking the standard deviation of a small, flat spectral interval close to the Raman line of the fluorescence-subtracted Raman spectrum. This fluorescence background was approximated by a polynomial fitting and subsequently subtracted from the raw Raman spectrum. Note that all graphs show the spectra without background subtraction.

3 | RESULTS AND DISCUSSION

3.1 | TRRS of Xanthoria elegans

In order to assess the suitability of the time-resolved setup for investigation of space-relevant biological samples, X. elegans was measured. Initially, Raman spectra were measured over a delay range of -300–1,950 ps with a coarse step size of 75 ps to determine the optimal delay parameters (Figure 4, left). The Raman spectrum at 150 ps delay is added to this overview figure to illustrate the main Raman lines. The shifting of the detector gate and the principle of TRRS are shown schematically in Figure 4 (right). The integration time was 10 s, and each spectrum is an average of 10 acquisitions. To prevent photochemical alteration of the lichen, the laser power of approximately 22 mW on the sample was reduced to approximately 5 mW by a neutral density filter in the beam path (Figure 3).

Between -300 and -75 ps delay, which is before the laser pulse, (Figure 4, left) only detector noise is recorded. This also indicates that the fluorescence is relatively short lived and completely decayed after 13.2 ns (76 MHz pulse repetition rate) and does not influence the measurement with the next laser pulse. Very weak Raman bands start to appear at 0 ps delay. The range [0 ps, 225 ps] shows the appearance of five Raman bands in total as well as an increase in broadband fluorescence intensity. From 300 ps delay, the fluorescence intensity decreases again, and at 375 ps delay, all Raman bands have disappeared (Figure 4, left and middle). From 225 ps (maximum fluorescence intensity) to 1,950 ps delay, the fluorescence intensity decayed by 88%. This decay depends on the fluorescence lifetime(s) of the fluorophore(s) in the examined system. In this case, the photoprotective pigment parietin is present (see next section). Apart from parietin, which exhibits a broad fluorescence band between 450 and 750 nm with a maximum at 505 nm,[55] numerous other components, for example, emodin, which has a similar structure to parietin (Figure 1), could contribute to the fluorescence background. Because the fluorescence...
spectra are relatively broad, and the examined biological system is highly complex, the exact determination of the individual fluorescent components is difficult and not part of this study.

The optimal delay time is defined by the highest SNR of the Raman lines. The exploratory measurements of Figure 4 showed that the optimum must be between 75 and 225 ps. Therefore, additional spectra were recorded at 125, 150, 175, and 200 ps delay (see Figure 5). The integration time was 10 s, and each spectrum is an average of 70 acquisitions to improve the SNR (see next section). Five Raman lines are clearly visible.

3.2 Comparison of gated and nongated Raman spectra of Xanthoria elegans

The gated (125 to 250 ps delay) and nongated Raman spectra at the same spot were compared, and this was repeated for four different measurement positions on the same sample. For both modes, an integration time of 10 s, 70 acquisitions, and a laser power of approximately 5 mW were used. The gated (175 ps delay) and nongated Raman spectra with the best SNR (fourth measurement position) are shown in Figure 6. For this particular spot, the fluorescence background relative to Raman signal was somewhat weaker than for the other spots.

The Raman spectra of both modes show five Raman lines at 927, 1,279, 1,369, 1,555, and 1,671 cm\(^{-1}\). Edwards et al.\(^{[11,40]}\) characterized the first Raman line at 927 cm\(^{-1}\) as out-of-plane-bending mode \(\delta\) (CH) of parietin whereas Fabriciova et al.\(^{[41]}\) assigned it to a ring-in-plane-bending vibration \(\delta\) (CCC) coupled to the stretching vibration of the methoxy group \(\nu\) (COC) of parietin. The second line at 1,279 cm\(^{-1}\) is attributed to the ring stretching vibration \(\nu\) (CC) by Fabriciova et al.\(^{[41]}\), which is coupled to the stretching \(\nu\) (CO)/\(\nu\) (COC), bending \(\delta\) (OH) and symmetric bending \(\delta\) (CH\(_3\)) modes of parietin. In the same study, the band at 1,369 cm\(^{-1}\) is characterized as symmetric in-plane-bending vibration \(\delta\) (CH\(_3\)) of parietin’s methyl group, whereas Edwards et al.\(^{[40]}\) assigned the line at 1,279 cm\(^{-1}\) to the ring stretching vibration (in plane) and the third line to the \(\nu\) (CO) stretching vibration of the phenolic groups. Furthermore, the aromatic C=C stretching vibration induces the Raman line at 1,555 cm\(^{-1}\).\(^{[40]}\) Fabriciova et al.\(^{[41]}\) described additionally a coupling to the carbonyl group \(\nu\) (C=O) and the bending mode \(\delta\) (OH). The fifth line at 1,671 cm\(^{-1}\) is due to the carbonyl stretching \(\nu\) (C=O)\(^{[11,40]}\) or the ring stretching vibration \(\nu\) (CC) coupled with the carbonyl stretching \(\nu\) (C=O) and the stretching mode \(\nu\) (C=C).\(^{[41]}\)

The main advantage of the time-resolved technique is that the majority of the fluorescence photons emitted by X. elegans are rejected by the detector gate. This is
supported by calculating and comparing the SNRs of the gated and nongated measurements for every measurement position. The SBRs in gated mode are compared with those in nongated mode and are improved in all four cases: by a factor of 3.2–22.8 (Spot 1), 1.9–23.0 (Spot 2), 4.1–24.0 (Spot 3), and 1.5–4.5 (Spot 4); Table 2. Higher values correspond to larger improvements due to gating and, thus, to a higher fluorescence rejection.

The SBR defines the level of fluorescence rejection but is not a direct measure for the improvement of the spectrum. We then considered the SNR, which defines the quality of the Raman signals and therefore allows a direct comparison between the quality of gated and nongated spectra. The SNR of every Raman line was calculated, whereby an SNR of at least 3 is regarded as a minimum for identification (Table 3).

The nongated Raman spectra of the first, second, and the third measurement positions do not show any clear Raman lines (SNR < 3.0), whereas the corresponding gated spectra show 4–5 Raman lines depending on the delay time. As a result, the SNR improves from 1.9 to 12.3 (Spot 1), from 3.2 to 11.3 (Spot 2), and from 2.9 to 6.4 (Spot 3). Although the third sample position shows only a small improvement of the SNR and not all five Raman lines were detected in the gated spectrum of the second measurement position, the time-resolved data reveal more Raman bands than the nongated measurements of the same spot. The fourth measurement spot is the only position where already in the nongated Raman spectrum all five Raman lines of parietin can be identified (SNR: 7.3–12.2), with roughly comparable SNR values for the gated and nongated modes.

### 3.3 TRRS of lunar regolith analogue material

In addition to the search for extraterrestrial life, mineralogical identification is another important goal in space research. Raman identification is a challenge when these minerals also fluoresce. This problem is analyzed based on the example of the fluorescent lunar regolith analogue material in the following section.

#### TABLE 2

Relative improvement of the gated SBRs compared to the nongated SBR of five different Raman bands of Xanthoria elegans [Colour table can be viewed at wileyonlinelibrary.com]

| Spot | Raman shift (cm\(^{-1}\)) | 125 ps | 150 ps | 175 ps | 200 ps | 225 ps | 250 ps |
|------|----------------|-------|-------|-------|-------|-------|-------|
| 1    | ~927           |       |       |       |       |       |
|      | ~1,277         | >12.0 | >10.5 | >3.5  | >6.9  | >3.2  |
|      | ~1,367         | >13.7 | >7.1  | >7.5  | >6.7  | >8.1  |
|      | ~1,557         | >15.5 | >9.6  | >8.7  | >5.5  | >7.0  |
|      | ~1,671         | >18.5 | >22.8 | >14.8 | >12.0 | >10.5 |
|      | ~1,277         | >16.4 | >10.5 | >6.8  | >8.2  | >8.6  |
|      | ~1,367         | >2.3  | >3.2  | >2.2  | >4.6  | >3.6  |
|      | ~1,557         | >2.7  | >4.4  | >2.3  | >2.1  | >2.3  |
|      | ~1,671         | >10.1 | >9.2  | >16.0 | >15.5 | >14.4 |
| 2    | ~927           |       |       |       |       |       |
|      | ~1,277         | >3.0  | >2.3  | >2.0  | >2.5  | >4.4  |
|      | ~1,367         | >1.9  | >3.2  | >2.2  | >4.6  | >3.6  |
|      | ~1,557         | >2.3  | >5.5  | >3.1  | >7.6  | >6.4  |
|      | ~1,671         | >1.1  | >12.0 | >17.3 | >18.1 | >23.0 |
|      | ~1,277         | >10.1 | >9.2  | >16.0 | >15.5 | >14.4 |
| 3    | ~927           |       |       |       |       |       |
|      | ~1,277         | >17.3 | >7.5  | >10.5 | >10.4 |
|      | ~1,367         | >24.0 | >18.3 | >18.2 | >11.6 |
|      | ~1,557         | >9.4  | >9.9  | >9.9  | >5.6  |
|      | ~1,671         | >9.4  | >11.3 | >8.4  | >5.3  |
| 4    | ~927           |       |       |       |       |       |
|      | ~1,277         | 2.6   | 3.0   | 2.3   | 2.0   |
|      | ~1,367         | 2.7   | 3.2   | 2.2   | 2.0   |
|      | ~1,557         | 4.5   | 3.8   | 3.2   | 2.1   |
|      | ~1,671         | 4.3   | 3.6   | 3.2   | 3.3   |

**Note.** The individual values represent the factors by which the SBRs of the gated Raman spectrum improve compared to the nongated spectrum; a value of 1 corresponds to no changes. White- or light gray background colored cells correspond to major changes. The > sign indicates that there are no Raman lines resolved in the corresponding nongated spectrum. SBR: signal-to-background ratio.
The integration time was 10 s, and each spectrum is an average of 10 acquisitions. All lunar regolith analogue material (LRS) spectra were recorded with a laser power of approximately 22 mW, because no alteration of the mineralogical sample had to be taken into account. As for the *X. elegans* measurements, first, Raman spectra were measured over a delay range of \(-300\)–\(1,950\) ps with a coarse step size of 75 ps to determine the optimal delay parameters. The detection of solely detector noise between \(-300\) and \(-75\) ps delay indicates the complete decay of the fluorescence intensity after 13.2 ns (Figure 7). From 0 ps delay, the fluorescence intensity increases until its maximum is reached at 225 ps. From 225 to 1,950 ps, the fluorescence photon intensity decreases by 90%. The decay depends on the fluorescence lifetime(s) of the individual fluorescent component(s) of...
the examined mineralogical mixture. The fluorescence of minerals often arises due to different impurities so-called activators or luminescence centers (e.g., Pr$^{3+}$, Nd$^{3+}$, Eu$^{2+}$, Dy$^{3+}$, Tb$^{3+}$, Ce$^{3+}$, Mn$^{2+}$, etc.) replacing cations in the host lattice.\[56\]

Like in the X. elegans measurements, weak Raman lines start to appear at 0 ps delay. At 75 ps delay various Raman lines at 478, 665, 820, 851, 962, 1,009, and 1,087 cm$^{-1}$ are visible and disappear again at 375 ps delay (Figure 7).

3.4 Comparison of gated and nongated Raman spectra of lunar regolith analogue material

The gated (125 to 200 ps delay) and nongated Raman spectra of three different measurement positions on the sample are compared. An integration time of 10 s and a laser power of approximately 22 mW were used for both measurement modes (gated and nongated). The average Raman spectra of 70 acquisitions in gated (175 ps delay) and the nongated modes of the second measurement position are shown in Figure 8.

Due to a relatively large laser spot size of approximately 120 μm on the sample, the Raman spectrum consists of a mixture of various minerals that are characterized by different Raman lines. Plagioclase is characterized by the breathing modes of the four-membered rings of tetrahedra\[57\] at 478 and 510 cm$^{-1}$, which are visible in the gated (175 ps delay) and the nongated spectra (Figure 8). The line at 478 cm$^{-1}$ in the nongated spectrum is disturbed by an instrumental artifact that was identified by the gated measurements at delay times larger 450 ps. Other Raman lines are not visible in the nongated spectrum. Here, the advantage of the time-resolved Raman measurement becomes apparent because further Raman lines are visible indicating that diopside, which is a pyroxene (665 and 1,009 cm$^{-1}$), and olivine (820 and 851 cm$^{-1}$) are present. Both Raman lines of pyroxene correspond to the vibrations of the SiO$_4$ tetrahedra in the silicate chains.\[58,59\] The band at 665 cm$^{-1}$ has been assigned to a Si–O$_b$–Si stretching mode and/or bending mode of the bridging (b) oxygen, which links SiO$_4$ tetrahedra to form the silicate chains.\[58–61\] The line at 1,009 cm$^{-1}$ is due to the Si–O$_{nb}$ stretching vibration of the nonbridging (nb) oxygen.\[58–61\] Both olivine bands arise from coupled symmetric and asymmetric Si–O stretching vibrations of the SiO$_4$ tetrahedra.\[62\] The line at 962 cm$^{-1}$, which is due to...
to the symmetric stretching mode of the phosphate ion PO₄³⁻ [63,64] indicates the presence of apatite. The Raman line at 1,087 cm⁻¹ could be assigned to the CO₃ stretching mode of a carbonate mineral, which is a constituent of the volcanic slag.

For comparison, first, the SBR of all Raman lines of every measurement position on the sample in gated and nongated mode is calculated and then compared. The SBR of the Raman line of plagioclase at 478 cm⁻¹ is not calculated in nongated mode, because the instrumental artifact disturbs the determination of the peak height. The SBR of the other lines in gated mode changes by a factor of 0.9–2.7 (Spot 1), 1.1–8.9 (Spot 2), and 0.9–5.2 (Spot 3) compared to the SBR in nongated mode (Table 4). The relatively large values of the second measurement position are due to the fact that the Raman lines at 665, 820, 851, 962, 1,009, and 1,087 cm⁻¹ are not visible in the Raman spectrum detected in nongated mode. Apart from that and compared to the lichen (see above), the SBR changes of the LRS sample are very small.

As mentioned above, not the SBR, but the SNR is a criterion for the quality of a Raman signal. Therefore, the SNR for every Raman line of every measurement position is calculated, whereby an SNR of at least 3 is considered to be the minimum for identification (Table 5).

The SNR of the first and third measurement position in nongated mode ranges from 8.9 to 34.3 and 3.0 to 53.4, respectively. The corresponding values in gated mode (Spot 1: 7.7–30.8 and Spot 3: 4.6–54.1) do not differ significantly. In these measurements, all Raman lines are visible in gated as well as in nongated mode (SNR > 3). Some Raman peaks have a slightly higher SNR in nongated mode, indicating that time-resolved detection offers no improvement in these cases. This can be attributed to the slightly higher noise of the gated ICCD detector, which is cooled to −11°C compared to −45°C for the nongated CCD detector. In the case of the second measurement spot, only two Raman lines at approximately 478 and 510 cm⁻¹ (plagioclase) are visible in the nongated spectrum. The gated spectra show an SNR improvement of the second line by a factor of 2.8. Because the line at 478 cm⁻¹ in the nongated spectrum is disturbed by the instrumental artifact, the corresponding SNR was not determined. Further signals of diopside

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**TABLE 5** The comparison of the gated and nongated SNR at various Raman line positions of LRS [Colour table can be viewed at wileyonlinelibrary.com]

| Spot | Raman shift (cm⁻¹) | 125 ps | 150 ps | 175 ps | 200 ps | Nongated |
|------|-------------------|--------|--------|--------|--------|----------|
| 1    | ~510 (pl)         | 30.8   | 23.1   | 28.6   | 24.7   | 34.3     |
|      | ~665 (di)         | 10.8   | 9.4    | 9.1    | 7.7    | 8.9      |
|      | ~962 (ap)         | 19.0   | 10.6   | 9.5    | 10.7   | 12.6     |
|      | ~1,009 (di)       | 14.2   | 11.1   | 10.8   | 11.4   | 13.7     |
| 2    | ~510 (pl)         | 29.0   | 28.1   | 30.6   | 29.4   | 10.4     |
|      | ~665 (di)         | 8.7    | 8.5    | 8.9    | 8.8    | 1.0      |
|      | ~820 (ol)         | 8.1    | 9.1    | 8.7    | 9.1    | 1.0      |
|      | ~851 (ol)         | 9.5    | 10.6   | 9.3    | 9.7    | 1.0      |
|      | ~962 (ap)         | 7.1    | 5.8    | 7.9    | 6.0    | 1.0      |
|      | ~1,009 (di)       | 10.8   | 6.3    | 9.5    | 7.7    | 1.0      |
|      | ~1,087 (CO₃)      | 4.6    | 7.6    | 4.9    | 4.4    | 1.0      |
| 3    | ~510 (pl)         | 40.3   | 40.0   | 54.1   | 47.2   | 53.4     |
|      | ~665 (di)         | 7.0    | 5.0    | 6.6    | 5.1    | 4.1      |
|      | ~820 (ol)         | 13.2   | 10.5   | 12.1   | 11.7   | 10.4     |
|      | ~851 (ol)         | 12.7   | 12.0   | 16.2   | 10.1   | 8.9      |
|      | ~962 (ap)         | 6.2    | 6.5    | 9.7    | 6.4    | 14.9     |
|      | ~1,009 (di)       | 7.1    | 6.7    | 8.7    | 6.4    | 15.2     |
|      | ~1,109 (?)        | 4.7    | 5.8    | 6.8    | 4.6    | 3.0      |

Note: A value of 3 is the minimum value for identification. White- or light gray-background colored cells correspond to a good signal-to-noise ratio (SNR). pl: plagioclase; di: diopside; ol: olivine; ap: apatite; CO₃: carbonate; ?: unidentified.
(665 and 1,009 cm\(^{-1}\)), olivine (820 and 851 cm\(^{-1}\)), apatite (962 cm\(^{-1}\)), and carbonate (1,087 cm\(^{-1}\)) become visible in the time-resolved Raman spectrum. The SNR improves at least by a factor of 4.4−10.8. In this case, the appearance of “new” Raman lines shows clearly the advantage of time-resolved measurements compared to nongated measurements.

4 | CONCLUSIONS

Gated and nongated Raman spectroscopy with an excitation wavelength of 440 nm was applied to one biological (\textit{X. elegans}) and to one mineralogical (lunar regolith analogue material) sample to evaluate a potential advantage of time-resolved (gated) Raman spectroscopy for the detection of space-relevant samples with a fluorescent background. This was realized by comparing the SBR and the SNR of time-resolved Raman spectra to nongated Raman spectra. The time-resolved technique yielded an improvement in all the spectra of \textit{X. elegans} where Raman lines of the photoprotective pigment parietin were detected. In most cases, the corresponding nongated Raman spectra showed no significant Raman signals. The improvement in the gated spectra compared with the nongated spectra is due to fluorescence suppression. This leads to significant improvement in the SBR values of the gated spectra compared to the nongated spectra.

For the LRS sample, those measurements spots that showed a good SNR in the nongated spectrum were not significantly improved by measuring in gated mode. However, spots that were dominated by fluorescence showed significant improvement in gated mode because of fluorescence suppression. Minerals such as plagioclase, pyroxene, olivine, apatite, and a carbonate mineral could be detected. Furthermore, the SBR changes of the LRS sample are very small compared with the SBR changes of the lichen, indicating a more effective fluorescence suppression through the time-resolved technique in the case of \textit{X. elegans}.

Although in this study, TRRS could not in all cases provide better results compared with nongated measurements, in most cases, a clear advantage could be demonstrated. This makes time-gated Raman detection a useful tool in future space missions. Future studies of a variety of biological and mineralogical samples could confirm the potential of this technique and serve as a database for a possible future space application. In recent years, first steps were made in miniaturizing a time-resolved setup (for instance, by Blacksberg et al.\textsuperscript{[65]}). This further increases the applicability of this technique for space missions.

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