Chapter 3

Time resolved Raman spectroscopy for depth analysis of multi-layered mineral samples

Jan-Hein Hooijschuur, Ingeborg E. Iping Petterson, Gareth R. Davies, Cees Gooijer and Freek Ariese

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Abstract
Time Resolved Raman Spectroscopy (TRRS) can provide subsurface information from multi-layered samples of transparent and translucent evaporative and silicate minerals up to several cm thick. Depth information was obtained using 3-ps pulsed laser excitation at 720 nm and a gated intensified CCD detector with stepwise increasing delay times. Blocks of different minerals were used as first, second or third layers and Raman spectra from deeper layers could be detected through 10 mm of translucent calcite and up to 40 mm of transparent halite crystals. Measurements by conventional confocal Raman, as well as Spatially Offset Raman Spectroscopy (SORS) were also successful in distinguishing different mineral layers. This study establishes the great potential for the use of Raman spectroscopy in future planetary exploration, where TRRS could be used as a non-invasive tool for profiling the (sub-)surface at millimetre-depth resolution.
3.1 Introduction

In the search for extra-terrestrial life Raman Spectroscopy (RS) has been recognised as an important component of the European ExoMars mission\(^{[20]}\). Key advantages of RS are that it provides chemical information non-invasively and water is not a source of interference. RS has already proven its applicability in geological and mineral studies\(^{[121,122]}\), and has been used extensively to study biological samples\(^{[123,124]}\).

(Raman) spectroscopic equipment will provide an elegant way to make the first selection of possible targets in the search for biomarkers. In combination with stand-off spectroscopic techniques, RS provides a non-invasive method and is a more rapid and practical approach than drilling and manipulating powdered samples. A planetary rover would not need to drive to a point of interest but could record Raman spectra up to a distance of 120 metres\(^{[16]}\).

One potential drawback with RS is that many organic and mineral compositions are fluorescent, which may cause strong interference to the Raman signal. One approach to overcome this is to apply longer laser wavelengths, so that fewer potentially interfering compounds can be excited. However, this will not completely remove the fluorescence background\(^{[97]}\). Additionally, the light scattering intensity and the quantum efficiency of CCD detectors decrease at longer wavelengths.

A second possible strategy is to utilize resonance RS (RRS), with an excitation wavelength that is in resonance with a chromophore in the target compound. This provides a selective Raman signal enhancement of several orders of magnitude, which may help to overcome background fluorescence and provides selectivity for the compound of interest over the solvent or matrix\(^{[25,26]}\).

A third strategy is to utilize Time Resolved Raman Spectroscopy (TRRS), based on the fact that Raman scattering and fluorescence emission happen on different timescales. Pulsed lasers and detection systems operating at the nanosecond (ns) timescale can be useful in avoiding long-lived luminescence of certain minerals\(^{[89]}\) but would not be very effective against an organic fluorescence background characterized by a much shorter lifetime\(^{[81]}\). For the study of fluorescent mineral samples, Blacksberg et al. (2010) have suggested the implementation of TRRS using a short laser pulse excitation and a streak camera with picosecond (ps) time resolution\(^{[85]}\) or a photodiode array detector with nanosecond time resolution\(^{[86]}\) to collect the Raman signal and reduce the fluorescence contribution. Kerr-gated TRRS with picosecond time resolution\(^{[84]}\) proved to be an effective technique to reduce the short-living fluorescence background in a Raman spectrum but requires high intensity pulsed lasers. In one of our previous TRRS studies, fluorescence suppression was achieved with an intensified CCD (ICCD) camera capable of a short (250 ps) detector gate in combination with low-energy 3-ps laser pulses from a 76-MHz Ti:sapphire laser system\(^{[87]}\).
Although TRRS was originally introduced to suppress the fluorescence contribution in a Raman spectrum or interference from ambient light in a stand-off Raman experiment[^105], an additional advantage of the technique is that it provides depth information[^82]. In a backscattering geometry, Raman photons originating deeper within the samples will reach the detector later in time.

Strongly scattering multi-layered samples are usually difficult to analyse with conventional continuous wave (CW) Raman instrumentation. In a backscattering collection geometry, the Raman signal is mostly derived from the surface, overwhelming the much weaker Raman signals from deeper layers. Focusing light deeper into non-transparent samples is usually not possible due to the scattering properties of the material in such cases. The time resolution of TRRS can retrieve information from the deeper layers of a sample by suppressing the Raman signal of the surface[^82,93]. Due to the random walk of photons in translucent media the 250-ps time resolution of our set-up allows depth profiling in materials at the millimetre scale[^94,97,125].

Depth information can also be extracted from the sample by the use of spatially offset RS (SORS)[^108]. This technique makes use of the fact that the point of excitation has a spatial offset with respect to the collection point of the Raman signals. When an offset of a few millimetres is applied, the intensity ratio between subsurface and surface signal improves. In this work, SORS as well as conventional confocal Raman using a commercial instrument will be compared to TRRS when applied to scattering multi-layered samples.

Figures 3.1a and 3.1b give a schematic view of the events of the TRRS set-up per laser pulse for a translucent, two-layered sample. At first a small fraction of the laser pulse reaches a trigger diode (represented in green) that activates the detector after a certain delay time. The detector then collects the signal over a 250-ps time frame, which is the minimum gate width of our system[^87]. The Raman signal can be collected at different delay times (typically at 25-ps increments), providing a depth profile of the sample. A short delay gives information predominantly about the surface (first layer signal, represented in blue) and a longer delay provides more selective information about the subsurface (second layer signal, represented in pink). In order to obtain a Raman spectrum exclusively of the deeper layer, any remaining contribution from the first layer can be removed by scaled subtraction of the short-delay spectrum. Since the photons are randomly scattered within the sample, the actual time that it takes for a photon to travel through the material is longer than expected based on the thickness and refractive index. We call this the photon retardation factor; it is a measure of the sample transparency and can be determined by measuring the delay corresponding with the onset of the second-layer signal. Monte Carlo modelling of scattering photon migration in a Raman experiment was presented by Everall and co-workers[^110].
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Figure 3.1: Schematic representation of depth profiling measurement related events over time, using short 3-ps laser excitation. The detector is synchronised to the laser pulse, initiated by the trigger and opens after an adjustable delay: in (a) short delay, the Raman signal of the first layer is collected; and in (b) longer delay, mainly the Raman signal of the second layer is accumulated with a smaller contribution of the first layer.

Our long-term goal is to assess the applicability of advanced Raman methods for the detection of biomarkers for planetary exploration. Environments that represent potential exobiology hotspots are suspected of containing or having contained water or ice at some time in the past. These are often extreme environments, having, in particular, high salt concentrations, and therefore much effort has focused on studying extremophiles, i.e., organisms that are capable of surviving such extreme environmental conditions[126]. In fact, living microorganisms or viable spores have been found inside halite crystals[70,71]. Microorganisms have also been found to grow on and in other kinds of minerals (epiliths), inside porous rocks (endoliths) or inside cracked rocks and minerals (chasmoliths)[56]. Via biotransformation (mobilisation or transformation of minerals) organisms can create a protective shelter[15], for example biotransformation of calcite[57].

The current focus of the search for life in our Solar System is Mars. The first billion years on Mars were warm and wet[34], providing ideal conditions for microbial growth as we understand them on Earth. Today Mars is cold, dry and the surface is continuously bombarded with energetic solar particles, cosmic rays and a high flux of ultraviolet radiation[127]. If microbial life existed on Mars or other sites of astrobiological interest, it may also have developed similar mechanisms to extremophiles on Earth for overcoming the harsh environmental conditions. If traces of these microorganisms are trapped below the surface of ice, salts or mineral layers, they may not be detectable on the surface by conventional spectroscopic techniques. There is therefore a need to look below the surface layers and detect biomarkers and other compounds of interest at depth. RS may provide a non-invasive alternative to drilling and crushing.
In this first step we explore the possibilities of TRRS for measurements through different layers of mineral; in the future we will elaborate on this work by examining biological samples. The mineral samples studied here represent a range from highly transparent (halite) to translucent (calcite) phases that are terrestrial hosts of microorganisms. For comparison, one set of two-layered samples will also be analysed by means of SORS and by conventional confocal RS.

3.2 Experimental
3.2.1 Samples
The following combinations of minerals were evaluated:

First layer materials
Calcite (CaCO$_3$), different blocks of 2.5, 5, 7.5, 10-mm thick: translucent;
Garnet (pyrope, Mg$_3$Al$_2$(SiO$_4$)$_3$), 4-mm thick: translucent;
Gypsum (CaSO$_4$·2H$_2$O), 6 and 11-mm thick: transparent, parallel to the β-axis;
Halite (NaCl), 40-mm thick: transparent.

Second layer materials
Calcite (CaCO$_3$), 10 mm: translucent (behind garnet, halite or gypsum);
Gypsum (CaSO$_4$·2H$_2$O), different blocks of 6 and 11-mm thick: transparent (behind calcite of different thicknesses).

Third layer material
Calcite (CaCO$_3$), 10 mm (behind garnet + gypsum).

Calcite, garnet and gypsum were obtained from our own mineral collection at the Vrije Universiteit Amsterdam. Halite was obtained from de Wereldwinkel, Amsterdam. Pyrope is used as a proxy for olivine that is widely found on Mars (both nesosilicates) [31]. Large quantities of gypsum are found on Mars[31]. Calcite is used as an example of translucent minerals and halite as model for transparent minerals. Both minerals are used to test the capabilities of the applied Raman spectroscopic methods.

3.2.2 Instrumentation
Figure 3.2 depicts the TRRS instrumental setup. A 532 nm, frequency-doubled Nd:YVO$_4$ laser (Coherent Verdi-V18, Santa Clara, CA, USA) operated at 9 W, pumps a tuneable Ti-sapphire laser that produces 3-ps pulses at a repetition rate of 76 MHz (Coherent Mira 900P). The laser has a fundamental wavelength range of 690-980 nm, and for this work, an excitation wavelength of 720 nm was used at a power of 610 mW at the sample. A Pellin Broca prism, pinhole and band-pass filter were used to clean up the laser light.
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Figure 3.2: Schematic representation of the time resolved Raman spectroscopy set-up where $L_n$ are lenses and $M_n$ are mirrors. For spatially offset Raman spectroscopy measurements, the 90° prism can be moved parallel to the sample surface so that the point of excitation is laterally offset relative to the point of collection.

The diameter of the laser beam at the sample was determined to be ~120 μm, by means of a WinCamD beam profiler (Dataray Inc., Bella Vista, CA, USA). For SORS measurements a small 90° prism can be moved parallel with respect to the sample surface. Via this prism, the laser spot can have a lateral offset of a few millimetres with respect to the collection point of the Raman signal. Lens 1 (diameter 25.4 mm and $f=50$ mm) collects the backscattered light and sends a parallel bundle through the long-pass filter. A dielectric stack long-pass filter, Semrock 735 AELP (Semrock Inc., Lake Forest, IL, USA), was used to block the reflected laser line and Rayleigh scattering. Lens 2 focused the backscattered Raman emission on the 70-μm entrance slit of a 50-cm single-stage spectrograph (SpectraPro, Acton, MA, USA).

Time-gated detection of the Raman spectra was performed using an intensified CCD camera (LaVision Picostar HR Picostar, Göttingen, Germany) with a microchannel plate with an operating potential difference of 850 V, at a temperature of 262 K, and triggered by a photodiode receiving a fraction of the laser output via a quartz plate. For comparative measurements, continuous wave detection was carried out using a non-gated CCD camera (model DV420-OE, Andor Technology, Belfast, UK) side-mounted to
the same spectrograph and operated at a temperature of 223 K. The intensity units of CW and TRRS detectors are not directly comparable.

Confocal Raman spectra at 785 and 532 nm were recorded using a Renishaw (Wotton-under-Edge, United Kingdom) InVia Reflex Raman microscope with a 300 mW, 785-nm diode laser in combination with the 1200 lines per millimetre (l/mm) grating and an 80 mW, 532-nm frequency doubled Nd:YAG excitation source in combination with an 1800-l/mm grating, and a Peltier cooled CCD detector (203 K). The instrument included a Leica light microscope with 5x air objective. The 521 cm\(^{-1}\) Raman shift of an internal silicon standard was used to verify the spectral calibration of the system. After baseline subtraction a Lorentzian peak fit was applied to determine peak centre and peak height. TRRS time delays are expressed relative to the laser pulse, and all spectra are shown with a y-axis offset for legibility.

3.3 Results and discussion
Using our setup at 720-nm excitation and with detection in either CW or time resolved mode, we first measured the spectra of various transparent and translucent minerals. These reference Raman spectra of the minerals are shown in figure 3.3a for CW RS; and 3.3b for TRRS measurements, and an overview of the peaks with their assignment is given in table 3.1. As expected, no Raman signals were obtained for the halite crystal.

![Figure 3.3:](image)

Raman reference spectra of gypsum (green, dot-dash), calcite (black, solid), garnet (red, dot) and halite (cyan, dash) at an excitation wavelength of 720 nm, 0.1 s exposure time and 1000 accumulations. Measured in (a) continuous wave Raman and (b) time resolved Raman mode. Spectra are vertically offset for legibility. The peak around 670 cm\(^{-1}\) in the CW spectra is an artefact of the laser.
Table 3.1: Overview of Raman peaks and assigned vibrations of the reference Raman spectra of calcite, garnet and gypsum. (Abbreviations: (a)sym = (a) symmetric; str = stretch; bend = bending; R = rotation).

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Raman shift (cm(^{-1}))</th>
<th>Assignment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcite</td>
<td>1086 (\nu_1 (\text{CO}_3) sym str</td>
<td>[128]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>711 (\nu_4 (\text{CO}_3) sym bend</td>
<td>[128]</td>
<td></td>
</tr>
<tr>
<td>Garnet</td>
<td>918 (\nu_1 (\text{Si-O})) sym str</td>
<td>[129]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>861 (\nu_1 (\text{Si-O})) asym str</td>
<td>[129]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>557 (\nu_2 (\text{Si-O})) sym bend</td>
<td>[129]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>368 (R (\text{SiO}_4))</td>
<td>[129]</td>
<td></td>
</tr>
<tr>
<td>Gypsum</td>
<td>1141 (\nu_3 (\text{SO}_4)) asym str</td>
<td>[130]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1008 (\nu_1 (\text{SO}_4)) sym str</td>
<td>[130]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>618 (\nu_4 (\text{SO}_4)) asym bend</td>
<td>[130]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>495 (\nu_3 (\text{SO}_4)) sym bend</td>
<td>[130]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>416 (\nu_2 (\text{SO}_4)) sym bend</td>
<td>[130]</td>
<td></td>
</tr>
</tbody>
</table>

Conventional Raman spectra were recorded with a confocal Raman microscope. The sample consisted of 2.5-mm thick translucent calcite as a first layer and gypsum as second layer. The spectra recorded at an excitation wavelength of 532 nm as shown in figure 3.4b have a fluorescent background, more than an order of magnitude stronger than when the Raman spectra are measured at the longer excitation wavelength of 785 nm, as shown in figure 3.4a. The inserts clearly illustrate how even a relatively weak fluorescent background (and the associated shot noise) can affect our ability to discern weak Raman peaks from deeper layers. In addition to the influence of excitation wavelength, figure 3.4 also illustrates the influence of the focal point for collecting Raman spectra. The focal point of excitation and collection coincide, regardless of the depth of the focal point. When the focus is at the surface, an optimal collection efficiency of Raman photons from the first layer is achieved. This results in a detectable but very weak gypsum peak at 1008 cm\(^{-1}\) in comparison with the very strong calcite peak at 1086 cm\(^{-1}\). By raising the sample stage 2.5 mm, the focal point would be deeper inside of the sample even though proper focusing is not possible in such scattering materials. The ratio of intensities between the 1008:1086 peak increases twofold at 785-nm excitation wavelength and sixfold at an excitation wavelength of 532 nm. The collection efficiency of first layer Raman photons shows a large decrease, whereas the intensity of the second-layer peak is about constant. In other words, the focus is more critical for the collection of Raman photons from the first layer, but if our aim is selectivity of the second layer, defocusing may actually be advantageous, as quantified in table 3.2.
**Figure 3.4:** Conventional Raman spectra of a sample composed of gypsum behind 2.5 mm of translucent calcite at an excitation wavelength of (a) 785 nm and (b) 532 nm recorded with a Raman microscope 1 s exposure time and 10 accumulations. The fluorescent background at 532 nm is about an order of magnitude higher than at 785 nm.

<table>
<thead>
<tr>
<th></th>
<th>Intensity second layer $1008\text{ cm}^{-1}$</th>
<th>Intensity first layer $1086\text{ cm}^{-1}$</th>
<th>Peak ratio second/first</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscope 785 nm focus at surface</td>
<td>3600</td>
<td>177000</td>
<td>0.020</td>
</tr>
<tr>
<td>Microscope 785 nm focus deep</td>
<td>3600</td>
<td>77000</td>
<td>0.047</td>
</tr>
<tr>
<td>Microscope 532 nm focus at surface</td>
<td>1800</td>
<td>410000</td>
<td>0.004</td>
</tr>
<tr>
<td>Microscope 532 nm focus deep</td>
<td>2100</td>
<td>82000</td>
<td>0.026</td>
</tr>
</tbody>
</table>

**Table 3.2:** Comparison of peak intensities and ratios of a sample composed of gypsum below a calcite layer of 2.5 mm using a conventional Raman microscope. The focus was either at the surface or the sample was moved 2.5 mm closer to the objective.

The same set of two-layered samples (gypsum behind different thicknesses of calcite) was studied with SORS and TRRS. The spectra recorded behind 10 mm calcite are shown in figure 3.5 (SORS) and in figure 3.6a (TRRS); the Ti:sapphire laser was used for excitation in both cases. Generally, the signal-to-noise ratio (S/N) of the CW-RS spectra is higher compared to the TRRS measurements. Using the CW-RS set-up but with no
lateral offset, the gypsum peak at 1008 cm$^{-1}$ showed a S/N of 35 (figure 3.5). With TRRS and a delay of 425 ps, the S/N was 22 (figure 3.6a). This can be readily rationalized since CW-RS collects all Raman signals per laser pulse whereas TRRS collects the signals over a 250 ps period only. However, for depth analysis the depth selectivity is a more important parameter.

**Figure 3.5:** (a) Overview and (b) detail of continuous wave, spatially offset Raman spectroscopy measurements at an excitation wavelength of 720 nm, 0.1 s exposure time and 1000 accumulations of a sample composed of gypsum behind a calcite block of 10 mm, with 0 mm and 5-mm spatial offset. Reference spectra of calcite (black, 1086 cm$^{-1}$) and gypsum (cyan, 1008 cm$^{-1}$) are also shown.

**Figure 3.6** (a) Time resolved Raman spectroscopy depth profile measurements of a sample composed of gypsum behind a calcite block of 10 mm. The calcite peak (1086 cm$^{-1}$) appears at 0 ps and gypsum (1008 cm$^{-1}$) at 300 ps. Recorded at an excitation wavelength of 720 nm, 1 s exposure time and 100 accumulations. Spectra are vertically offset for legibility. (b) Relation of calcite first layer thickness and time of photon retardation of the gypsum peak (1008 cm$^{-1}$) back and forth through the calcite blocks.
In figure 3.5, the effect of applying a spatial offset is shown. Without an offset, the characteristic peak of gypsum at 1008 cm\(^{-1}\) is detectable but very weak in comparison with the very strong calcite peak at 1086 cm\(^{-1}\). Upon applying a 5-mm offset, the ratio of intensities between the 1008:1086 peaks increases threefold. This is the result of a relatively larger decrease in intensity of the first layer calcite peak (1086 cm\(^{-1}\)) compared to the relative small decrease of the second layer gypsum peak (1008 cm\(^{-1}\)). These findings fully agree with the SORS principles as described by Matousek and co-workers\(^{[108]}\). The ratios determined from the peak heights are shown in table 3.3.

### Table 3.3: Comparison of peak intensities and ratios between SORS at different spatial offsets and TRRS at different delay times, determined for a sample composed of gypsum behind a calcite layer of 10 mm.

<table>
<thead>
<tr>
<th></th>
<th>Intensity second layer 1008 cm(^{-1})</th>
<th>Intensity first layer 1086 cm(^{-1})</th>
<th>Peak ratio second/first</th>
</tr>
</thead>
<tbody>
<tr>
<td>CW-SORS 0 mm offset</td>
<td>2800</td>
<td>264000</td>
<td>0.011</td>
</tr>
<tr>
<td>CW-SORS 2.5 mm offset</td>
<td>1600</td>
<td>52000</td>
<td>0.031</td>
</tr>
<tr>
<td>CW-SORS 5 mm offset</td>
<td>900</td>
<td>32000</td>
<td>0.028</td>
</tr>
<tr>
<td>TRRS at 225 ps</td>
<td>1400</td>
<td>324000</td>
<td>0.004</td>
</tr>
<tr>
<td>TRRS at 325 ps</td>
<td>3800</td>
<td>157000</td>
<td>0.024</td>
</tr>
<tr>
<td>TRRS at 425 ps</td>
<td>2300</td>
<td>70000</td>
<td>0.033</td>
</tr>
<tr>
<td>TRRS at 525 ps</td>
<td>1900</td>
<td>45000</td>
<td>0.042</td>
</tr>
</tbody>
</table>

The TRRS spectra were also measured at an excitation wavelength of 720 nm; no spatial offset was applied. In figure 3.6a, the TRRS spectra of the same sample, gypsum behind 10 mm of calcite, are shown as a function of the delay time. At short delay times - up to 275 ps - only the calcite peak at 1086 cm\(^{-1}\) can be observed; as expected, its intensity increases with longer delay times. After 300 ps, the gypsum peak at 1008 cm\(^{-1}\) becomes visible, indicating a distinction in composition of the different layers in the sample. Gypsum could be detected through up to a 10-mm layer of translucent calcite. The retardation of the photons is caused by multiple scattering events in the translucent mineral.

Detection after 300 ps implies that the net speed of light (\(v\)) back and forth through the calcite sample is 0.67\(\cdot\)10\(^8\) m/s\(^{-1}\) as the thickness of the sample (\(d\)) multiplied by 2 is divided by time (\(t\)) corresponding to equation (3.1). The theoretical speed (\(v_t\)) of light through calcite, where the speed of light through vacuum (\(c_0\)) is divided by the refractive index (\(n\)) of calcite (\(n = 1.486\)) according to equation (3.2) is 2.02\(\cdot\)10\(^8\) m/s\(^{-1}\). One can conclude that there is a factor of 3.03 difference between the calculated and the theoretical speed of light. The photon retardation factor is calculated by dividing the results of equation (3.2) by equation (3.1).

\[
v = \frac{2d}{t}
\]  

(3.1)
\begin{equation}
\nu_t = \frac{c_0}{n}
\end{equation}

The TRRS measurements were repeated with different thicknesses of the calcite first layer, and the corresponding retardation times of the second-layer photons before detection are shown in figure 3.6(b). Although the general trend of the graph shows linearity of the photon retardation time, the data points are scattered. For example, the photon retardation of the 7.5-mm thick block of calcite is much lower than would be expected based on interpolation of the other sample points. The explanation of this variation is the heterogeneity of the natural samples, which results in variation in the degree of transparency and hence their scattering potential.

To further evaluate the potential of TRRS for depth analysis of mineral samples, we investigated different combinations of minerals as first and second layer material. In figure 3.7a spectra of calcite behind a 40-mm block of transparent halite are shown. The Raman photons of the calcite (1086 cm\(^{-1}\)) became visible after a delay time of 550 ps. The maximum intensity was observed after 775 ps. Because the Raman signals are recorded in backscatter mode, the Raman photons travelled a minimum distance of 80 mm back and forth through the mineral to become visible after 550 ps. The calculated photon retardation factor of halite (\(n=1.544\)) is 1.33, which implies that there is much less contribution of scattering from the transparent halite sample than in the case of the translucent calcite sample discussed above.

Similarly, when TRRS is applied to a sample that has a first layer of 11-mm transparent gypsum and calcite as second layer, the Raman photons of the calcite (1086 cm\(^{-1}\)) appear after 150 ps, as shown in figure 3.7b. The maximum intensity was observed after 325 ps. The calculated photon retardation factor of gypsum (\(n=1.525\)) is 1.34, which again implies that there is less scattering by the gypsum sample compared to the calcite.
For the two-layered sample of calcite behind 4 mm of translucent garnet, the calcite peak at 1086 cm\(^{-1}\) appears after 75 ps. The calculated photon retardation factor of garnet (\(n=1.800\)) is 1.56, which implies that there is less scattering by the garnet sample compared with the calcite but more scattering compared with gypsum and halite. An overview of the different photon retardation factors calculated for two-layered samples is shown in table 3.4.

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Photon retardation factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcite</td>
<td>3.03</td>
</tr>
<tr>
<td>Garnet</td>
<td>1.56</td>
</tr>
<tr>
<td>Gypsum</td>
<td>1.34</td>
</tr>
<tr>
<td>Halite</td>
<td>1.33</td>
</tr>
</tbody>
</table>

**Table 3.4:** Overview of calculated photon retardation factors.

In figure 3.8, the spectra of a three-layered system are shown. The first layer is a 4-mm thick piece of translucent garnet, the second layer consists of a 6-mm piece of transparent gypsum and the final layer is calcite. In the first 25 ps, only the Raman spectrum of garnet (861 and 918 cm\(^{-1}\)) is observed. After a delay time larger than 50 ps, the Raman spectrum of gypsum (1008 cm\(^{-1}\)) starts to contribute to the detected Raman signal. Finally, at a 175-ps delay time, the calcite signal (1086 cm\(^{-1}\)) appears in the Raman spectrum. To illustrate the contribution of all three layers, the Raman spectrum after 300-ps delay time is also shown. Relevant Raman peaks from all three mineral layers are clearly resolved.

**Figure 3.8:** Time resolved Raman spectroscopy depth profile measurements of a sample composed of calcite behind a gypsum block of 6 mm in turn behind a 4-mm piece of garnet. The pure spectrum of garnet (861 and 918 cm\(^{-1}\)) is observed at 0 ps. Gypsum (1008 cm\(^{-1}\)) appears at 50 ps, and the calcite peak (1086 cm\(^{-1}\)) appears at 175 ps. Recorded at an excitation wavelength of 720 nm, 0.1 s exposure time and 1000 accumulations. Spectra are vertically offset for legibility.
The results presented in the previous text establish that RS provides a potentially strong method to distinguish the presence of different mineral layers. In this initial study, we used calcite, gypsum, garnet and halite as examples of different types of minerals. CW-RS can be used for the identification of pure mineral samples, as shown in figure 3.3a. However, when minor peaks from a different mineral are also observed in a spectrum, this could be due to a second material hidden below the surface or a minor impurity homogeneously distributed over the sample. With a single measurement, one cannot distinguish between these two cases. When a spatial offset of a few mm is applied, however, the ratio of the peak intensities can be used as an indication of different mineral layers, as shown in the SORS spectra of figure 3.5 and quantified in table 3.3.

When RS is used in combination with confocal microscopy, the same phenomena can be observed as by changing the lateral offset in SORS. As shown in figure 3.4, the intensity ratio of the peaks changes when the focal point is deeper into the two-layered sample. This is an indication of two distinct mineral layers with different mineral composition. However, with confocal Raman microscopy, we could only measure Raman spectra through a maximum of 2.5-mm thick calcite layer. When SORS or TRRS were applied, the second layer could be detected through up to 10 mm of calcite.

The influence of fluorescence on Raman spectra is illustrated by comparing the Raman spectra of two different excitation wavelengths. Fluorescence can be a drawback of Raman spectroscopy as demonstrated in figure 3.4b. Although the fluorescence of the calcite sample was not particularly strong and did not completely overwhelm the Raman spectrum measured with a 532-nm excitation source, the background noise was dramatically increased compared to the 785-nm excitation source.

An appropriate method for depth analysis of mineral samples via RS is TRRS, as indicated in figures 3.6-3.8. By adjusting the delay time of the detector, direct structural information can be obtained from layered mineral samples. The method has proved effective in detecting a second mineral behind a first layer of 10 mm of translucent calcite and 40 mm of transparent halite. The three-layered sample with 4 mm of translucent garnet as a first layer, 6 mm of transparent gypsum as second layer and calcite as third layer proved the depth resolution of the method to be at millimetre range for both transparent and translucent minerals.

In SORS measurements, peaks from both layers are always visible, and only the ratio of the peak intensities differs when a spatial offset is applied. Similar ratio changes were observed when changing the sample position relative to the focal point in conventional Raman measurements. A much larger ratio difference of the peak intensities can be achieved by TRRS (table 3.3). At short delay times, a strong first layer Raman signal is observed, with little to no contribution of Raman signals from deeper
layers. At longer delay times, the intensity of the Raman signals of the first layer starts to decrease, and the peak intensities of the second layer increases until there is little to no contribution of Raman signals of the first layer remaining. This makes TRRS a selective tool for distinguishing different layers of minerals, and the time delay is a direct measure of the depth of the subsurface material. In contrast, the S/N ratios tend to be better with CW measurements\textsuperscript{[125]}.

3.4 Conclusions
Applying TRRS measurements to different combinations of mineral layers provided excellent results for both transparent and translucent minerals, demonstrating the analytical strength of the method. The unique possibility to selectively obtain information from deeper layers shows that the TRRS is potentially a strong analytical tool for planetary exploration by rover or stationary landers when no active sampling mechanisms can be employed.

Using an excitation wavelength of 720 nm also helps with fluorescence reduction compared to using a green wavelength, for example. However, in future studies on organic biomarkers with resonance excitation, we expect much higher levels of fluorescence interference. Previous studies with our set-up at 460-nm excitation showed that the time gating considerably reduces fluorescence\textsuperscript{[97]}. The instrumental setup used in this study is equipped with a tuneable Ti:sapphire laser source for the red and near-infrared spectral region, with second and third harmonic options for blue and ultraviolet output. An optical parametric oscillator with tuneable output over the visible range or other flexible excitation source could provide additional information by selective resonance of specific (bio)markers.

In an astrobiological perspective, the TRRS set-up shows great potential. Detection of different layers of minerals is possible through clear crystals up to 40 mm in thickness and up to 10 mm of a translucent mineral. Most natural rocks will be finer grained and multi-mineralic, leading to lower effective detection depths. The TRRS technique, however, does appear to offer the possibility for the non-invasive detection of extremophiles living in and on minerals, and ongoing research will elaborate on this subject.

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