COMPARING RAMAN SIGNAL STRENGTHS OF BIOMARKERS AND MINERALS MEASURED BY A MULTI-WAVELENGTH RAMAN SYSTEM Jie Wei, Alian Wang and Kathryn Connor, Dept of Earth and Planetary Sciences and McDonnell Center for Space Sciences, Washington University in St. Louis, (jiewei@levee.wustl.edu, St. Louis, MO, 63130, USA).

Planetary Raman Spectroscopy: pros and corns

Raman spectroscopy probes fundamental vibrations of molecules that produce finger-print spectral patterns with sharp peaks. Raman spectra can be acquired non-invasively, non-destructively and fast, thus is suitable for landed surface explorations on planetary bodies. On the other hand, Raman scattering phenomenon is intrinsically weak. It requires carefully crafted optical configurations with high efficiency optical and opto-electronic components, in order to obtain high Raman signal strength and to reduce instrument noises. The most mature, field-tested (in the Atacama Desert) Raman system MMRS/CIRS (supported by PIDDP, MIDP, ASTEP, MatISSE) , uses a CW green laser (532 nm) for excitation. With a spectral coverage of 532-675 nm, it takes the full advantage of the most mature and efficient optical and opto-electronic technologies in the visible region (lasers, optics and CCD detectors) [1, 2]. It has hitherto the simplest configuration and the highest technical readiness level.

In terrestrial geological applications of Raman spectroscopy, one of the major limitations comes from possible fluorescence interference from the analyzed samples. The fluorescence emission can be produced from bio-genetic species that were trapped in porous rocks or soils (e.g., clays). They can also be generated from the electronic transition modes of rare earth elements (e.g., REE–enriched minerals), or in some cases from transition metals.

We conducted three types of studies to address the potential fluorescence interference in planetary Raman spectroscopy. First, to measure the fluorescent properties of a broad range of extraterrestrial materials, such as lunar samples, Martian meteorites, chondrites, achondrites), which resulted a conclusion of non-to-minimum threat to the Raman signals from a CW green Raman system [3, 4]. Second, to develop SERDS and SSE technologies, based on which we demonstrated that it is feasible to extract Raman signal with acceptable S/N when in rare cases the fluorescence background is high [5]. Third, because changing excitation wavelength was one of the proposed paths to overcome fluorescence problem, we compare Raman signal strengths measured on a same set of samples, with different excitation wavelengths, using a state-of-art multi-wavelength Raman spectrometer, i.e., a comparison of the best possible technical practices to understand the gains and losses.

Samples – Minerals and Biomarkers

Five standard minerals were selected on the basis of being commonly seen in igneous and sedimentary formations. Six organic compounds were selected on the basis of their appearance as biomarkers in ancient terrestrial rocks and in meteorites. Biomarkers are organic compounds with structures and chemistry that are diagnostic for biological processes; some of them are stable under harsh environmental conditions in long period of time. All biomarkers are pure chemicals. All minerals are natural, in crystalline form, with large sizes (> 1 cm diameter) and flat-cut surface, which ensured good sampling geometry during the Raman measurements of different excitation wavelength.

A state-of-art multi-wavelength Raman system

The goal of this study is to compare the Raman signal strengths from a same set of samples using different excitation wavelengths. In order to make a reasonable comparison, *a Raman system capable of employing multi-wavelength for excitation, while keeping the same optical configuration for all wavelengths, but using the optimized optical components for each wavelength is desirable to use*. The inVia Raman system from Renishaw Company satisfies these three requirements. It was purchased by MFRP-PME fund and WUSTL fund, and was installed by the end of 2012.

This WUSTL-inVia system allows five excitation wavelengths at 785 nm, 633 nm, 532 nm, 442 nm, and 325 nm. Figure 1a shows the four lasers and the guiding optics on the back of inVia main optics. Figure 1b

Figure 1a. 5-λ inVia Raman system at WUSTL

Figure 1b. One set of optical path before slit (exchangeable optics)

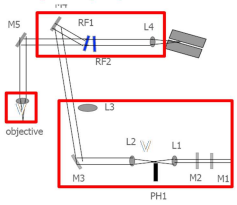
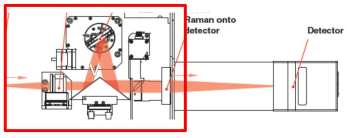


Figure 1c. One set of optical path after slit (exchangeable optics)



and 1c show one set of optical path before and after the slit. InVia has three sets (IR, Vis, UV) of mirrors, lens, objectives and five sets of filters and gratings (all op-

timized to a targeting excitation wavelength) that are exchangeable when selecting a specific excitation wavelength, with an auto-alignment program. The exchangeable optical elements are marked by red-rectangles in Figure 1b & c. This system represents the same optical configuration for all five excitation wavelengths and the best optical practice (technology and alignment) for each of them, except using the same CCD detector. Thus it enables a sound Raman signal strength comparison.

Methodology for a sound comparison

We split the Raman excitation path from Raman collection path; normalize each of them separately, then to make the comparison.

To facilitate the comparison in excitation path, Raman measurements were carried out with the same laser power (5 mW) on the all samples. The same objective lens (Leica 50X LW, NA=0.50) was used for IR (785 nm) and green (532 nm), but a Thorlab LMU-NUV40x (NA=0.5) was used for UV (325 nm). The same exposure time (1 s) and number of co-add (1) were used for all measurements. Figure 2 shows such a comparison for the mineral of feldspar. The Raman signal strength from green excitation is about 100x times of that from UV excitation.

Figure 2. Comparison of Raman signal strength of a feldspar sample generated using five laser lines for excitation on a commercial Raman system (inVia, by Renishaw Company) that has separated & optimized optical channels for UV, Vis, and IR-Raman.

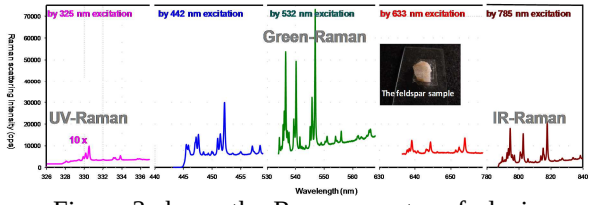
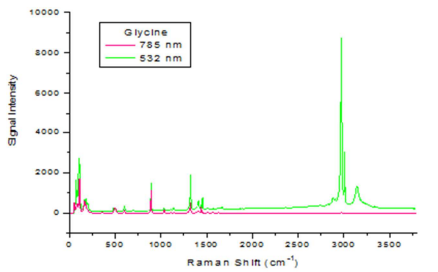


Figure 3 shows the Raman spectra of glycine excited by 532nm and 785 nm lasers. A clear difference between the two spectra is low signals at large Raman shits (near 3000 cm^{-1}) with 785 nm laser. This is caused by the low CCD quantum efficiencies at longer wavelengths [6]. Below Raman shift of 1000 cm^{-1}, the two spectra are comparable.

Figure 3. Raman spectra of glycine with 532 nm and 785 nm lasers



The comparison in collection path is more complicate. Note the same CCD camera is used for recording Raman signals that has different Quantum Efficiency (QE(λ)) in different Raman spectral ranges that corresponding different excitation wavelength λ_n. In addition, the anti-reflection coating on lens, grating, and mirrors for UV (although they are the best technical practice) will cause further reduction of UV optical efficiency. We will use a standard white-light source with known emission intensity I (λ) in full spectral range to obtain the overall throughput/response of collection path at different excitation wavelength $TR_{\lambda n}$ (λ), and to use it normalizing the collected Raman signal. We have just received the calibration source that will enable us to do so. The results will be presented at the conference.

Table 1 gives the positions of the strongest peaks at the laser wavelengths of 785 nm and 532 nm from a preliminary measurement. CCD's response changes the most intense peaks from high Raman shift peaks with a 532 nm laser to low Raman shift peaks with a 785 nm laser.

Table 1. Positions of the most intense peaks at 785 nm and 532 nm

Compounds	785 nm	532 nm
β-carotene	1155	1155
Cholesterol	1436	2866
Glycine	892	2972
Myristic acid	1295	2881
N-acetyl-L-phenylalanine	1004	1004
Octadecane	1295	2881
Calcite	1086	1086
Feldspar	512	512
Olivine	854	856
Pyroxyne	665	665
Quartz	464	464

The pair of peaks at 1155 and 1515 cm-1 due to C-C and C=C stretching[7] are most intense in β-carotene Raman spectra at both laser wavelengths. For cholesterol (a biosynthesized lipid molecule), myristic acid (a common saturated fatty acid) and octadecane (an alkane hydrocarbon), the CH stretching peaks around 2900 cm^{-1} are most intense at 532 nm, while CH_2 bending mode peaks are the strongest at 785 nm. Glycine is the smallest amino acid, and one of the 20 amino acids commonly found in proteins. Its Raman spectra at 532 nm also has the strongest peak due to the CH stretching vibration; while at 785 nm, the highest peak is due to the C-C stretching vibration [8]. A benzene structure is contained in another amino acid, N-acetyl-L-phenylalanine, which has been found in meteorites. The ring breathing vibration of the benzene structure[9] contributes to the highest peak in its Raman spectra at both 785 nm and 532 nm. The strongest peaks in the Raman spectra of the minerals are the same for both 532 nm and 785 nm lasers.

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References: [1] Wang et al. (2003), *JGR*, 108, 5; [2] Wang et al. (2014) 2nd IPM, #1090; [3] Wei et al. (2014), 2nd IPM, #1112; [4] Wei et al. this LPSC; [5] Lambert et al. (2014) 2nd IPM, #1136; [6] McCreery (2000), *Raman spectroscopy for Chemical Analysis*, John Wiley & Sons; [7] Marshall C. and Marshall A (2010), *Phil.Trans. R. Soc.A*, 368, 3137-3144; [8] Rosado et al. (1998), *Vib. Spectrosc.* 16, 35; [9] Gelder et al. (2007), *J. Raman Spectrosc.*, 38, 1133.