Fluorescence Detection Limits of Organic and Biological Targets Using a Compact Spectrometer from 5 m Distance. M. J. Egan[1], A. K. Misra[1], S. K. Sharma[1], T. E. Acosta-Maeda[1], J. N. Porter[1], M. W. Sandford[1] and M. N. Abedin[2], [1]Hawai'i Institute of Geophysics and Planetology, Univ. of Hawai'i at Mānoa, Honolulu, HI 96822, USA; [2]NASA Langley Research Center, Hampton, VA 23681, USA. eganm@hawaii.edu

Introduction: Fluorescence spectroscopy is a valuable analytical tool in the search for biosignatures within the solar system because of its ability to quickly identify organic compounds. Organic compounds have fluorescence lifetime decays of 100 ns or less, while other categories of compounds have luminescence lifetime decays of micro or milliseconds[1]. In addition, the quantum yield of fluorescence is quite high, reaching up to 95% quantum yield in some compounds like xanthene fluorescent dyes (e.g. Rhodamine 6G), while competing active-sensing spectroscopy techniques such as normal Raman spectroscopy are 10^7 less efficient[2]. Since the quantum yield of fluorescence is so high, laser-induced fluorescence measurements can be made of very small quantities of material or samples at great distances. Because of these properties, two laser-induced fluorescence instruments were selected to be part of the Mars 2020 rover, namely SuperCam and SHERLOC. These fluorescence spectrometers are equipped with differing excitation wavelengths (e.g. 532 nm for SuperCam; 248.6 nm for SHERLOC) and so will be able to excite different compounds into their first excited singlet state and thereafter record different regions of the electromagnetic spectrum. As the launch date for the world's first fluorescence spectrometers to leave Earth approaches, the question naturally arises, what quantities of biologically important molecules would need to be extant on the surface of another planetary body in order for a remote-sensing fluorescence spectrometer to detect it? The purpose of this work is to quantify the amount of material required to produce a fluorescence spectrum and thereby bound the conditions in which a future mission might find life or its derivatives.

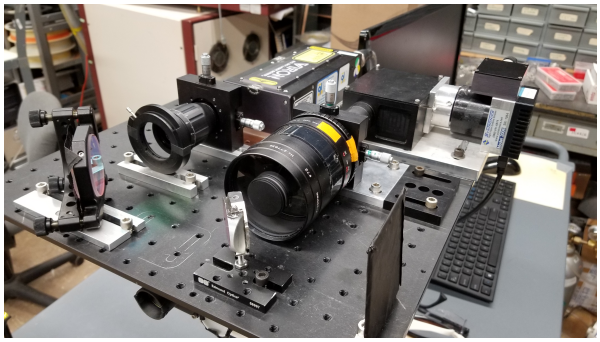

Fig. 1 – The compact Raman and fluorescence spectrometer developed at the University of Hawai'i used in this work.

Experimental Method: A depiction of the instrument used to collect fluorescence spectra is presented in Figure 1. Fluorescence spectra were obtained by illuminating various samples with a 532 nm pulsed Nd:YAG laser, operating at 12.5 mJ per pulse and 20 Hz. Samples of Rhodamine 6G in water, and dissolved organic matter in ethanol were placed 5 meters from the collecting optic. The area of the circular laser spot incident upon the sample was 59.87 mm^2; however, only laser light superimposed upon an image of the entrance slit at the sample location (i.e. 9.76 mm^2) yielded fluorescent photons that would contribute to the recovery of the desired fluorescence spectrum. The effective sampling area, or the area where the laser spot and entrance slit coincide, was 6.93 mm^2. Therefore, the effective laser power utilized in this experiment was 1.45 mJ per pulse. Spectral light was collected by a 3" Bower mirror lens, focused through a 50 µm entrance slit, collimated by an additional lens, diffracted into two traces by a holographic transmission diffraction grating, and recorded by an intensified charge-coupled device (ICCD) manufactured by Synctronics. Each spectra presented in this abstract was the result of 600 laser pulses.

Samples: Pure Rhodamine 6G was purchased from the Aldrich Chemical Company, and bio-mass was obtained by dissolving green leaf plants in ethanol. The resulting precipitant consisted of a variety of dissolved organic matter (DOM), most of which was chlorophyll. Dilution series of Rhodamine 6G and DOM were prepared in water and ethanol solvents, respectively, ranging in concentration from one part per million (ppm) to one part per billion (ppb). Each concentration in each dilution series were prepared in 20 mL quantities and placed within glass vials.

Results and Discussion: Rhodamine 6G is a xanthene dye renowned for its extremely high fluorescence quantum yield, reaching 95% QY in water[3]. Since Rhodamine 6G has such a high QY, many researchers have used it as a fluorescence standard throughout the years and so its fluorescent properties are well defined. Upon determining the limit of detection of Rhodamine 6G using the diffraction grating spectrometer utilized in this study, the limit of detection of a variety of other compounds may be estimated by comparing quantum yield values published in the literature. Figure 2 illustrates the fluorescence signal obtained after applying the experimental method described above. Two features are present in the spectra

shown in Fig. 2, namely the fluorescence peak of Rhodamine 6G centered at ~ 558 nm, and three convolved Raman peaks of water located between 640 and 650 nm. The Raman peaks of water[4] shown here are a superposition of the symmetric stretching mode (ν_1), the anti-symmetric stretching mode (ν_3), and an overtone of the symmetric bending mode (2 ν_2). The combined Raman and fluorescence spectra of Rhodamine 6G in concentrations of 1 ppm and 100 ppb are not present in Fig. 2 because acquiring said spectra, even with a single laser pulse accumulation, saturated the ICCD. Lastly, a Rhodamine 6G solution in concentration of 100 parts per trillion was prepared, but identification of the fluorescence peak of Rhodamine 6G was not possible given the defined experimental conditions.

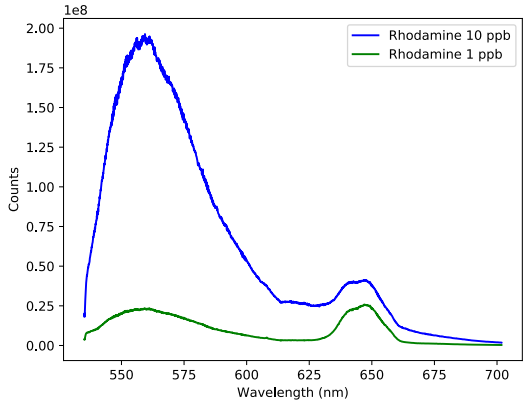

Fig. 2 – The fluorescence spectra of dilute Rhodamine 6G solutions (10 ppb and 1 ppb) in water.

Likewise, the fluorescence spectra of dilute DOM solutions were measured and are presented in Fig. 3. In Fig. 3, the chlorophyll fluorescence emission[5] can be seen as the broad feature occurring at 669 nm. The remaining spectral features arise from the Raman scattering of laser light by ethanol.[6] The key Raman features present in Fig. 3 are the CH_2 symmetric stretch, CH_3 symmetric stretch, and CH_3 anti-symmetric stretch appearing in Fig. 3 as a triplet spectral feature centered at ~ 630 nm, the OH stretching motions that give rise to the broad peak centered at ~ 645 nm, and the medley of CC, CO stretches and CH_2, CH_3 bending motions that produce peaks in the 540 to 580 nm region of the spectrum. The most dilute concentration of DOM that could be measured by the experimental method outlined earlier was the 100 ppb sample.

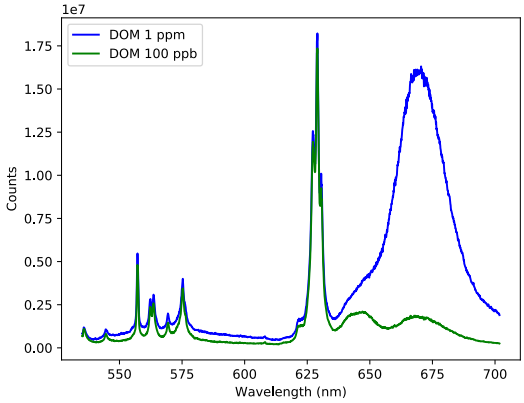

Fig. 3 – The fluorescence spectra of dilute dissolved organic matter (DOM) in ethanol (1 ppm and 100 ppb).

Summary: Fluorescence spectroscopy is an important analytical tool in the search for life and organics in the solar system because the technique's high quantum yield relative to other forms of active-sensing spectroscopy, its ability to recover fluorescence spectra even from great distances, and its ability to differentiate between different classes of molecules by quantifying the fluorescence lifetime decay time. As the advent of laser-induced fluorescence in non-terrestrial environments approaches with the launch of the Mars 2020 rover, the authors believe it's time to quantify the limit of detection of fluorescent proteins, polyaromatic hydrocarbons, etc… in order to constrain the quantities required for detection and thus the environmental conditions that would need to be prerequisite in order for remote-sensing fluorescence instrument to detect them. The results of this investigation will be presented at the conference.

Acknowledgments: This work has been supported by NASA PICASSO and the Mars 2020 mission grants.

References: [1] Misra, A. K. et al. (2016) *Astrobiology*, 16 (9), 715-729. [2] Bhartia, R. et al. (2010) *Appl. Environ. Microbiol.*, 76 (21), 7231-7237. [3] Bindhu, C. V. et al. (1999) *Mod. Phys. Lett. B*, 13 (16), 563-576. [4] Walrafen, G. E. (1964) *J. Chem. Phys.*, 40 (11), 3249-3256. [5] Broyde, S. B. (1966) *Biophys. J.*, 6, 353-366. [6] Mammone, J. F. et al. (1980) *J. Phys. Chem.*, 84, 3130-3134.