Standoff Time-Resolved Fast Fluorescence of Organics and Amino Acids

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Introduction:

Detection and characterization of organics, proteins, and amino acids play a significant role for NASA's search for past and present life in astrobiological targets. Amino acids and nucleobases are of particular interest due to their involvement in biological processes and as basic constituents of life as we know it. Proteinogenic and non-proteinogenic amino acids and nucleo bases have been reported in Martian meteorites, carbonaceous chondrites, and in samples returned by NASA's Stardust spacecraft from comet 81 P/Wild 2 [1-4]. Characterization of key proteins and amino acid signatures such as tryptophan, tyrosine, phenylalanine, glycine, β-alanine, γ-amino-n-butyric acid, L-alanine, L-Glutamic acid, and β-aminoisobutyric acid supports future rover investigations on astrobiological targets. Fluorescence excitation of individual aromatic residues of folded proteins, such as tryptophan, tyrosine, and phenylalanine, will aid in identifying amino acid interactions that lead to active protein native states [5].

In the Mars 2020 mission, the SuperCam instrument will be able to remotely analyze targets using Raman spectroscopy, Laser-Induced Breakdown Spectroscopy (LIBS), and Time-Resolved Fluorescence Spectroscopy (TRFS), without the need for sample manipulation or treatment. Among the three techniques, the Raman spectroscopy provides unambiguous identification of organics, inorganics and biological components [6]. Both LIBS and fluorescence spectroscopies have much higher sensitivity for detecting very low concentration of biological components. Raman and fluorescence spectroscopies provide non-destructive detection of bio-molecules in comparison to LIBS. Laser-induced fluorescence (LIF) spectroscopy is based on measuring the optical emission of molecules that have been excited to higher electronic energy levels by absorption of electromagnetic radiation from a monochromatic pulsed laser source. Fluorescence signals are several orders of magnitude higher than Raman signals, which make the LIF technique suitable for detecting a minute amount of bio-molecules in a large search area. Planetary minerals containing transition metal and rare-earth ions produce fluorescence spectra when excited with UV and visible lasers. This fluorescence can in some cases overlap with the fluorescence spectra of biogenic and organic compounds. However, the fluorescence decay time or lifetime of biogenic and organic compounds is much shorter (<100 ns) as compared to the fluorescence decay time of μs to ms of the transition metal ions and rare-earth ions in minerals and rocks. This feature is exploited in the time resolved fluorescence spectroscopy to detect a biological target. Here, we present data on various amino acids, proteins, and hydrocarbons using remote time-resolved fluorescence spectroscopy.

Instrumentation:

For this study a combined remote Raman+LIBS+TRFS system utilizing a 532 nm Nd:YAG pulsed laser and an 8 inch collection telescope was used. Remote TRFS spectra were measured in the 350-850 nm spectral range from a distance of 9 m using 10 mJ/pulse of laser power for excitation. For planetary exploration, a compact, portable remote Raman+LIBS+TRFS system using 2.5 inch telescope has also been developed under the Mars Instrument Development Program [4, 7]. The prototype is capable of measuring mineral targets up to 50 meters away using Raman and fluorescence spectroscopies and has a LIBS range of 10 m. The laser spot diameter is adjustable between 0.3 mm and several cm. Good quality spectra can be obtained at shorter distances of 10 m or less within 1 s integration times. The system includes time gating capabilities therefore is able to distinguish between organic and inorganic fluorescence, as well as identify atmospheric gases between the system and target.

Samples and Methodology:

We measured all of the proteinogenic amino acids (21 L-amino acids and glycine kit, Fluka), sarcosine, and several polyaromatic hydrocarbons, such as anthracene. Samples were placed and measured through sealed glass vials. The lower Raman cross-section of glass allowed for sample analysis without interference from the glass sample holder [8]. Remote fluorescence measurements were recorded from 9 m distance with 1 s detection time (equal to 15 laser shots excitation) using a 10 mJ/pulse laser power output, and 7 mm laser spot size on the target. The time-resolved fluorescence spectra were measured with sequential 10 ns gate widths.

Results and Discussion:

Time resolved fast fluorescence measurements were made on 31 amino acids, proteins, and hydrocarbons. Exponential decay was witnessed in all fluorescent molecules with a duration of less than 100 ns (fast fluorescence). The rate of this decay is sensitive to environmental variables that quench the fluorescence. Strong fluorescence signals with very short lifetimes were observed in most amino acids. In addition, sharp peaks corresponding to spontaneous Raman signals were also observed concurrently. Proteins contain three aromatic amino acid residues: tryptophan, tyrosine, and phenylalanine. Through various mixtures, these contribute to their intrinsic fluorescence.

Figures 1 and 2 show time-resolved fluorescence spectra of L-Tryptophan and L-Phenylalanine respectively, over sequential 10 ns time intervals with a 1 s integration time. The inelastic Raman scattering caused by the vibrational modes of molecules has a life-time of $\sim 10^{-13}$ s resulting in Raman signals that are simultaneously observed along with the bio-fluorescence signal in nanosecond time frames.

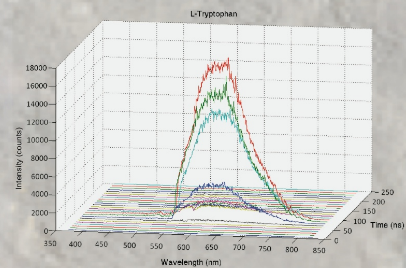

Figure 1: L-Tryptophan exhibits a wide fluorescence band at ~620 nm. Aromatic amino acid fluorescence arises from free electrons inside the aromatic rings. Acosta-Maeda et al., [4] show complementary Raman spectra to the fluorescence spectra presented here.

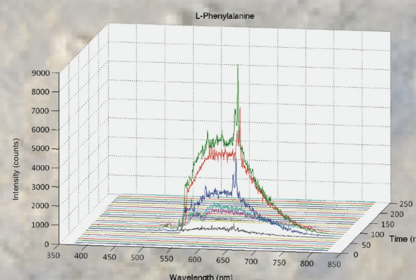

Figure 2: L-Phenylalanine exhibits a wide fluorescence band at ~615 nm and several Raman peaks with strongest Raman line at 635.8 nm. It is an aromatic amino acid and exhibits Raman lines representative of CH rings arising from ring-breathing vibrations in the mono-substituted aromatic compounds and from aromatic C-H stretch vibrations.

Figures 3-8: Organics, proteins, amino acid, and hydrocarbon spectra in order of strongest to weakest fluorescence. Increasing fluorescence yields a rough relationship with fluorescence band positions centering closer to 630 nm and masking most to all of the Raman lines, and weaker fluorescence band positions centering closer to 600 nm and increasingly visible Raman lines. In proteinogenic amino acids (precursors to proteins), simpler chain structures tend to yield higher fluorescence. Polar uncharged side chain amino acids serine, threonine, asparagine, and glutamine exhibit successively weaker fluorescence bands that are centered around 630 nm. The additional NH_2 molecules present in asparagine and glutamine yield strong Raman bands unmasked by the fluorescence. CNH ring structures and hydroxyl radicals with only one bond connection to the remainder of the molecule appear to yield higher fluorescence signals than those which exhibit additional bonds to OH, possibly due to the increased ring vibration freedom. This is evident when comparing proline spectra (CNH ring) to tyrosine (OH ring structure with an hydroxyl radical bonded). Proline is also unique in that it is the only amino acid whose side chain is connected to the protein backbone. An exception to this is the highly fluorescent tryptophan, which includes adjacent CNH and CH ring structures. This is due to the specific ring structures in the tryptophan functional groups that enable resonating electrons to reach higher energy states upon excitation.

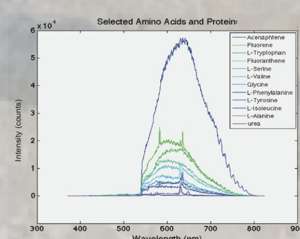

Figure 3: The relative intensity of fast fluorescence of various proteins and amino acids under the same experimental conditions. Acenapthene Benzene exhibited the strongest fluorescence completely masking the Raman lines. Fluorene, L-Tryptophan, Fluoranthene, and L-Serine exhibited the next highest fluorescence signals, with Fluorene showing strong Raman lines at 582.2 nm and 635.3 nm. Urea displayed the lowest fluorescence signal.

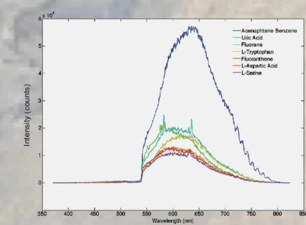

Figure 4: The strongest fluorescence signals are displayed. A large concentration of C-H bonds may be responsible for acenaphtene benzene's strong fluorescence.

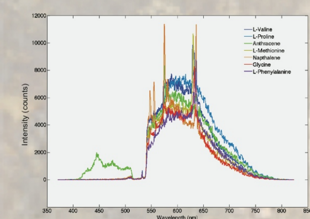

Figure 5: The fluorescence bands are roughly centered around 630 nm. In the case of anthracene, the strong fluorescence signals are also observed on the anti-stokes side.

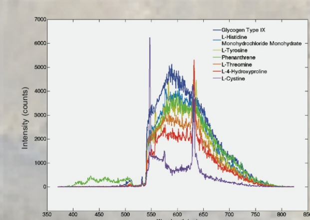

Figure 6: Fluorescence band centered around 600 nm. Cystine displays prominent Raman lines at 545 and 635 nm unmasked by fluorescence. Phenanthrene displays fluorescence below 532 nm which indicates a two photon fluorescence process.

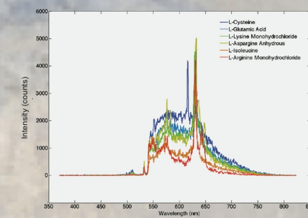

Figure 7: Broad fluorescence band centers are positioned around 600 nm with distinctive Raman lines at 635 (3044 cm^{-1} Raman shift) and 632 (2989 cm^{-1} Raman shift) nm representatives of CH stretching modes in the rings.

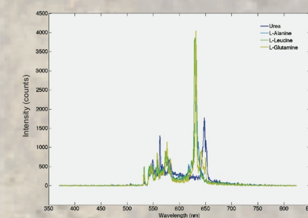

Figure 8: The weakest fluorescence signals correspond with amino acids with hydrophobic side chains containing two NH2 molecules for alanine and leucine. Urea and glutamine also contain two NH2 molecules bonded to the molecule spine.

Conclusions:

We have determined that time-resolved fast fluorescence can be used to detect organics. Characterization of amino acids, proteins, and other organic material provides the necessary biomarker signatures to successfully distinguish between fluorescent inorganics and biogenic materials on planetary surfaces. Additional expansion of combining fluorescence spectroscopy with Raman is necessary to uniquely identifying structures within organic molecules and to distinguish natural mineral mixtures with organics. Fluorescence spectra of meteorites, volatile ices, and hydrous minerals containing organics would greatly enhance the scientific value of fluorescence data and may serve as a useful laboratory database for comparative analysis with in-situ fluorescence spectra on Mars and future astrobiological targets such as Europa. Few biological components naturally fluoresce so identification would be easy since discriminating between matrix and biological components should be obvious. Daytime detection of amino acids and nucleo-bases from a distance of 9 m has been demonstrated using time-resolved fluorescence spectroscopy that is well suited for planetary exploration applications, requires no sample preparation or collection, and provides non-destructive rapid detection. The SuperCam instrument on MARS2020 mission will be able to locate biological and organic materials using its fast fluorescence mode and identify the chemical structure using remote Raman spectroscopy.

Acknowledgment:

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