

Life Detection Beyond Earth: Laser-Based Mass Spectrometry for Organics Detection on Solar System Objects

Andreas Riedo^{a,b*}, Nikita J. Boeren^{a,b}, Peter Keresztes Schmidt^a, Marek Tulej^a, and Peter Wurz^{a,b*}

Abstract: The detection and identification of the building blocks of life, from amino acids to more complex molecules such as certain lipids, is a crucial but highly challenging task for current and future space exploration missions in our Solar System. To date, Gas Chromatography Mass Spectrometry has been the main technology applied. Although it has shown excellent performance in laboratory research, it has not yet been able to provide a conclusive answer regarding the presence or absence of a signature of life, extinct or extant, in space exploration. In this contribution we present the current measurement capabilities of our space prototype laser-based mass spectrometer for organics detection. The developed mass spectrometer currently allows the detection and identification of small organic molecules, such as amino acids and nucleobases, at sample concentrations at the level of femtomole mm^{-2} , using the same measurement protocol. The latter is highly relevant to space exploration, since with the instrumentation in use so far only one class of organics can be measured with one instrument configuration.

Keywords: Laser desorption mass spectrometry · Life detection · LIMS · Organics · Space exploration



Andreas Riedo received his PhD in Physics in 2014 from the University of Bern, Switzerland. In 2016 he received an SNSF fellowship that allowed him to conduct his research in Astrobiology at the Leiden University, The Netherlands. He extended his stay with an MCSA fellowship for another two years before he moved in 2019 to the Free University Berlin after receiving the Einstein fellowship. In 2020 he moved to

University of Bern, received his *Venia Docendi* in 2022, and is currently appointed as project leader for a LIMS instrument to be deployed on a CLPS mission within NASA's Artemis program.



Nikita J. Boeren received her BSc in Analytical Chemistry from the University of Applied Sciences Leiden and an MSc jointly from the University of Amsterdam and Vrije Universiteit Amsterdam, The Netherlands. As a PhD candidate at the University of Bern, her research focuses on the detection of biosignatures using the ORIGIN setup for *in situ* space exploration targeting Mars, Europa, and Enceladus.



Peter Keresztes Schmidt received his BSc and MSc in Chemistry at the ETH Zürich, Switzerland. He is currently working as a PhD student in Bern, Switzerland, where he is developing a new LIMS instrument for *in situ* studies on the lunar surface.



Marek Tulej received a PhD in Physical Chemistry from the University of Basel, in 1999. After his postdoctoral period at Paul Scherrer Institute (PSI), he joined the University of Bern in 2008 as an instrument scientist for space missions, including Phobos-Grunt, Marco Polo-R, Luna-Resurs, and JUICE.



Peter Wurz has a degree in electronic engineering (1985), an MSc and a PhD in Physics from Technical University of Vienna, Austria (1990). He has been a postdoctoral researcher at Argonne National Laboratory, USA until 1992, after which he joined the University of Bern and became a full Professor of physics in 2008. From 2015–2022 he was the head of the Space Science and Planetology division, and since 2022 director of the physics institute. He has been Co-I and PI for many science instruments for space missions of ESA, NASA, ISRO, CNSA, Roscosmos, and JAXA.

1. Introduction

With the two Viking spacecraft that landed on Mars in the 1970s,^[1] humanity began the challenging endeavour of detecting and identifying signatures of life in our Solar System. The landers were equipped with highly sophisticated payloads at that time, which included Gas Chromatography Mass Spectrometric (GC-MS) systems for molecular signature identification.^[2–5] Unfortunately, *in situ* measurements of Martian surface material using the Viking science instrumentation did not provide conclusive evidence of the presence of life. Since then, other exploration mis-

*Correspondence: PD Dr. A. Riedo, E-mail: andreas.riedo@unibe.ch, Physics Institute, University of Bern, Bern, CH-3012 Bern, Switzerland

sions have been or will be launched to search for life in our Solar System. NASA's Curiosity^[6] and Perseverance rovers^[7] are currently operating on the surface of Mars to better understand habitability conditions and search for life, and are the best equipped rovers ever operated in space science. In addition, the Perseverance rover is currently collecting sample material and sealing it in tubes, in preparation for later collection and transfer to Earth for detailed laboratory analysis as part of the Mars Sample Return Mission.^[8–9]

There are different categories into which signatures of life can be grouped. The Mars 2020 Science Definition Team has defined six promising groups, ranging from isotope fractionation to macroscopic signatures visible to camera systems.^[10,11] Building blocks of life, such as amino acids or lipids, are arguably a prominent category of life signatures. Their detection could indicate the presence of life as we know it. Consequently, the astrobiology community has been pushing for detection capabilities for these molecules on past, present, and future missions.^[12,13] So far, GC-MS systems have been widely used in exploration missions because of their measurement capabilities,^[14] e.g. L and D amino acids can be distinguished with the appropriate GC column, and their excellent performance in laboratory research. However, since the Viking missions to Mars, the GC-MS systems have not provided conclusive evidence of the presence of life. As a result, the space science community is looking for alternative and sensitive measurement technologies for future use.

Since the beginning of 2000, the Mass Spectrometry Group of the Institute for Space Research and Planetary Sciences at the University of Bern has been working on the design and in-

vestigation of laser-based mass spectrometers for the chemical (elements, isotopes, and molecules) analysis of samples for their potential application on future exploration missions.^[15,16] In this contribution, we highlight the current measurement capabilities of our ORganics Information Gathering INstrument (ORIGIN),^[17] which has been designed for *in situ* detection and identification of various groups of organics. ORIGIN is a Laser Ablation Ionisation Mass Spectrometry (LIMS) system that operates in the laser desorption mode to gently desorb organic molecules from sample surfaces. Based on the current measurement capabilities, several international groups have shown interest in using this technology on future missions, ranging from signature detection in the Venusian cloud to lipids on the Martian surface.

2. ORIGIN – Laser-based Mass Spectrometry

2.1 System Description

ORIGIN is a LIMS system operating in the laser desorption mode (highly reduced irradiance compared to the laser ablation mode) for the sensitive detection and identification of organic molecules. The system is described in more detail in previous publications,^[17–19] so only a brief overview of the measurement principles is given here. The schematics and operating principle are shown in (Fig. 1). The system consists of a miniature reflection-type time-of-flight mass analyser^[16] coupled to a pulsed nanosecond laser system (Nd:YAG, $\tau \sim 3$ ns, $\lambda = 266$ nm, laser pulse repetition rate of 20 Hz, actively Q-switched). The simple beam delivery system placed on an optical table directs the laser pulses to the lens system which focuses the laser beam through the mass

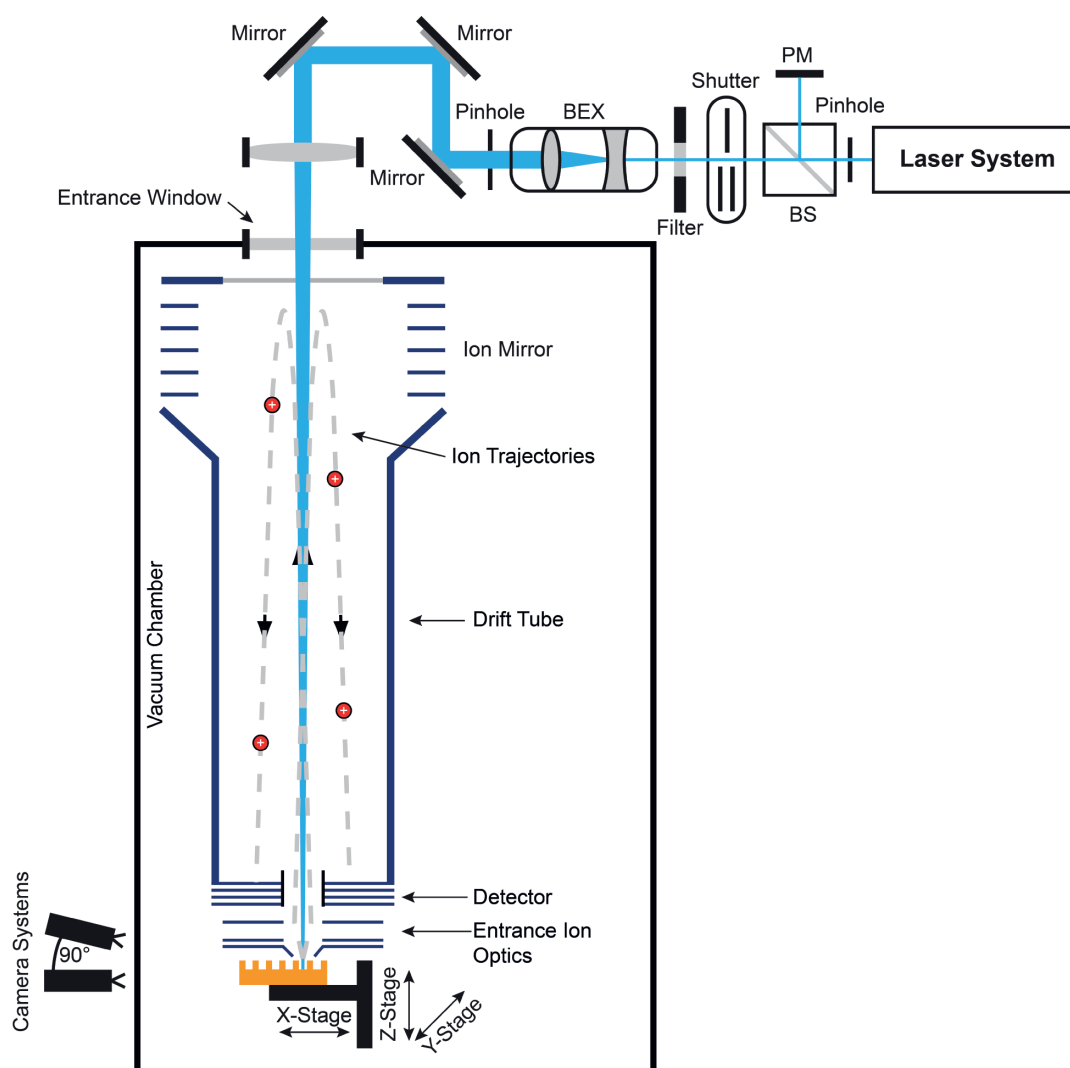


Fig. 1. Operating principle and schematics of the ORIGIN LIMS setup used for organics detection and identification.

analyser onto the sample surface to spot sizes of about 30 μm in diameter. Pulse energies in the range of about 1–5 μJ (at the surface) are typically applied in mass spectrometric studies, resulting in laser irradiances in the order of MW/cm^2 . Each laser pulse gently desorbs and ionises the analyte of interest, allowing the positively charged species to enter the entrance ion optical elements of the mass analyser. The species are accelerated and confined towards the drift tube, reflected at the ion mirror towards the multichannel plate (MCP) detector system^[20] through the drift tube a second time. The species arrive at the detector system sequentially according to their mass-to-charge ratio (Time of Flight (TOF) measurement principle). Read-out-electronics with a sampling rate of up to 2 GS/s record the TOF spectrum (length of 20 μs) for each laser pulse, which is then stored on the host computer for post processing. Software packages written in-house are used for subsequent data analysis.^[21] Empty TOF spectra (*i.e.* no mass peak detected above the noise floor, typically 6 sigma) are not considered for analysis. The sample holder is placed on an XYZ stage to allow for accurate positioning of the holder below the mass analyser aperture. Two cameras mounted orthogonally allow for visual feedback of the sample holder position. In Fig. 2 the visual imaging of the sample holder together with the entrance ion optics of the mass analyser from one of the cameras is shown. The bright white spot visible below the mass analyser corresponds to the focus of the laser beam and the induced plasma (here laser ablation conditions).

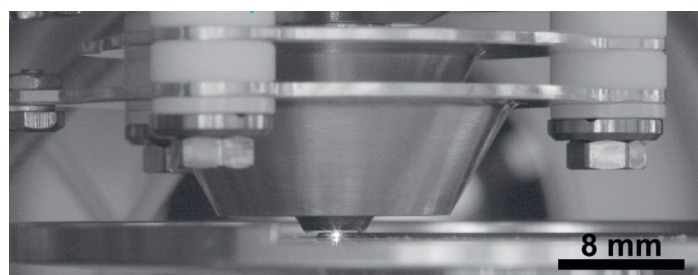


Fig. 2. *In situ* imaging during LIMS measurements. The bright spot below the mass analyser aperture corresponds to the focused laser beam.

2.2 Measurement Protocol

Typically, 1 μL of sample analyte is drop cast into a cavity (0.2 mm \times \varnothing 3 mm) of a sample holder. It is important to note that in contrast to MALDI no matrix is present within the cavity. Prior to integration into the vacuum chamber, the sample holder is placed on a clean bench (ISO5) where the water or other solvent containing the analyte of interest can evaporate in a clean environment. The sample holder with the remaining organic residue film is then placed in the vacuum chamber, the chamber is evacuated, and once a low enough pressure is reached ($\sim 5 \times 10^{-7}$ mbar) the mass spectrometric measurements are initiated. Each cavity is typically spot-wise investigated over 40 surface positions (can be adapted to the scientific needs), considering the inhomogeneous distribution of the residue film (see Fig. 3). A Python control package conducts the measurements autonomously according to user input.

3. Detection and Identification of Organics

3.1 Amino Acids

After the design and manufacturing phase of ORIGIN, the first measurements were conducted in 2019 on up to 20 biotic and abiotic amino acids, drop cast into cavities of a stainless steel holder at various concentrations (1 μL drop cast of 100–1 μM solutions, corresponding to 14–0.14 pmol/mm^2 , or 100–1 pmol/g ice), mixtures thereof, and contaminated with NaCl salt.^[17] The afore-

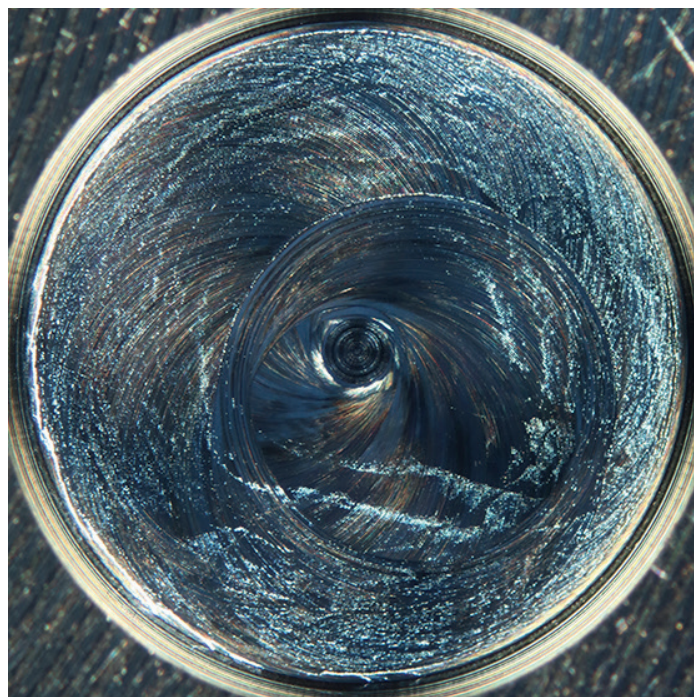


Fig. 3. Optical microscopy image of a sample cavity containing adenine (1 mM concentration, in solution). Adapted from Boeren *et al.* ref. [19].

mentioned gram of ice corresponds to a sample received during a mission on an icy moon, *e.g.* Europa Lander mission.^[12] The measurements showed that each amino acid can be identified in the mass spectra by its simple and unique fragmentation pattern induced by the laser-molecule interaction. The current setup does not allow differentiation between isomers, *e.g.* iso-leucine and leucine, or measurement of the enantiomeric excess (L/D). The top panel of Fig. 4 shows a mass spectrum of aspartic acid (Asp, 100 μM , in solution, 100 pmol/g ice, 14 pmol/mm^2) measured using the typical measurement protocol. The recorded mass spectrum shows three distinct mass peaks of Asp, allowing the amino acid to be reliably identified. When distributed over the 40 surface positions, variations in intensities are observed, as expected, due to the inhomogeneous distribution of the molecule ‘film’ over the cavity. However, the ratio between the fragments remains almost constant (within one sigma, see Fig. 2 in Ligterink *et al.* 2020),^[17] allowing an identification by fragment ratio analysis, comparable to element isotope analysis. Consequently, this information can be used to feed a database that allows the simulation of amino acid patterns. The lower panel of Fig. 4 shows such a simulation of Asp, simulating the three major fragments observed for Asp. It is planned to consolidate the software routine (not yet finalised) for post-analysis of the presence and abundances of species within a more complex mixture by fragment ratio analysis and least-square fitting. In addition, the first large measurement campaign carried out on up to 20 amino acids using the ORIGIN setup, has shown that there is a linear correlation between fragment intensity and amino acid concentration. In Fig. 5 the linear correlation between the drop cast concentration and one of the major fragments (at mass m/z of 110) of histidine (His) is shown. This is extremely valuable information as it allows a rough estimation of the abundances of the molecules detected. It should be noted that such a correlation can only be derived for thin films or low concentrations of organics, as we expect to receive on *e.g.* Mars or the ice moons Europa and Enceladus.^[12,13] Above a certain concentration, resulting in a thick layer of organics, we expect a saturation effect due to a maximum limit in desorbed sample material per laser shot using our current measurement protocol.

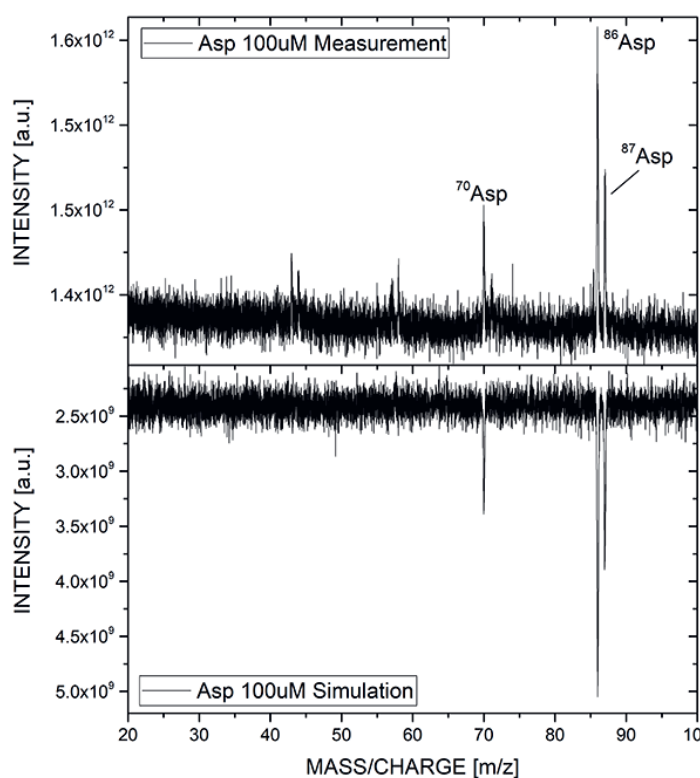


Fig. 4. Top: measured mass spectrometric pattern of aspartic acid (Asp), drop cast with a concentration in solution of 100 μM . Bottom: simulated spectra of Asp.

For space exploration missions, an instrument is typically designed to detect and identify only one class of molecules, for example amino acids. This minimises the complexity of the instrument and the associated risks in manufacturing and operation, as well as the costs overall. The disadvantage of this approach is that it is pre-selective, *i.e.* other molecule classes may not be detected. This is particularly true when using the current gold standard in organic analytics, the GC-MS instrumentation, where the selected column defines which class of molecules can be detected *in situ* on the planetary object. Therefore, and after exploring the detection of amino acids in more detail, *e.g.* using network analysis to separate matrix and signal from molecules,^[23] it was logical for our team to explore how the measurement protocol developed for amino acids could be applied to different and more complex organic molecules.

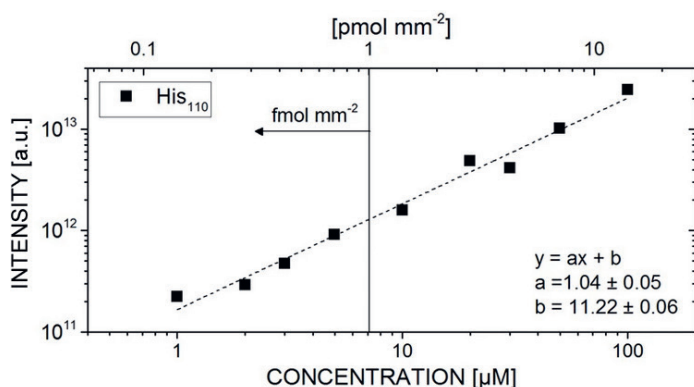


Fig. 5. Correlation between amino acid concentration and recorded fragment intensity of the amino acid histidine. Here, one of the major fragments of His with mass m/z of 110 was used for the correlation. Image taken and adapted from Ligterink *et al.* ref. [17].

3.2 Polycyclic Aromatic Hydrocarbons (PAHs)

So far, the measurement protocol has allowed the detection of Polycyclic Aromatic Hydrocarbons (PAHs),^[22] which are representative of more complex molecules, lipids,^[18] and more recently, nucleobases,^[19] the latter two being linked to life as we know it. PAHs do not have the same importance for life as amino acids or lipids, as they are abundant in the Interstellar Medium (ISM), however they could be precursor molecules important for life, see discussion in Kipfer *et al.* and references therein or Ehrenfreund *et al.*^[22,24] In Fig. 6 the successful detection of four different PAHs (anthracene ($\text{C}_{14}\text{H}_{10}$), pyrene ($\text{C}_{16}\text{H}_{10}$), perylene ($\text{C}_{20}\text{H}_{12}$), and coronene ($\text{C}_{24}\text{H}_{12}$), 100 μM in solution (100 pmol / g ice, or 14 pmol mm^{-2}), measured at 4 μJ laser pulse energy) using ORIGIN and the identical measurement protocol as for amino acids is shown. In contrast to amino acids, the parent (for anthracene, perylene, and coronene) or protonated (pyrene) mass spectrometric peaks were detected directly, allowing their easy identification. Of the five PAHs analysed, coronene had the clearest mass spectrum by means of fragmentation. The robust aromatic ring structures of PAHs and the improved coupling with UV wavelengths clearly enhance the laser desorption process. For the investigated PAHs, limits of detection (3 sigma) of only a few tens of fmol mm^{-2} were observed, and, similar to amino acids, the abundance can be derived from the detected peak signals (see Figs. 7 and 8, respectively, in Kipfer *et al.*).

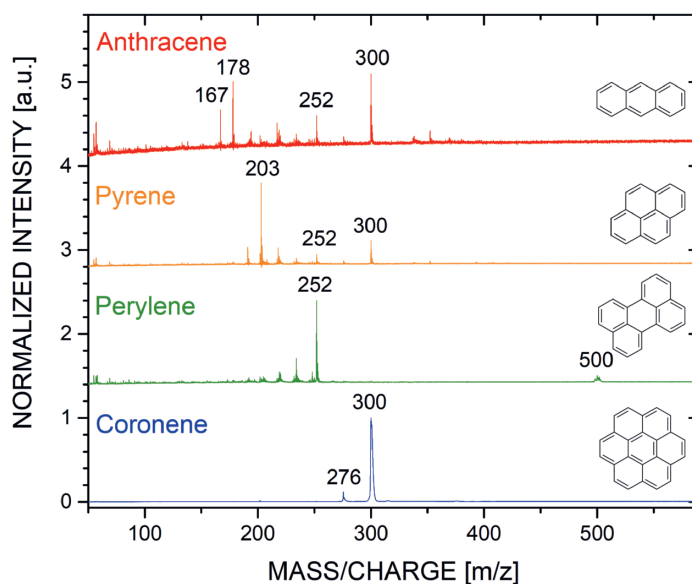


Fig. 6. The PAHs anthracene, pyrene, perylene and coronene (100 μM , in solution) were successfully detected and identified using the same measurement protocol as for the amino acids. Image adapted from Kipfer *et al.* ref. [22].

3.3 Lipids

In Boeren *et al.*^[18] six different lipids were studied in detail, including cholecalciferol, phyllo-quinone, menadione, 17 α -ethynylestradiol, α -tocopherol, and retinol. In Fig. 7 the mass spectra of α -tocopherol (430.71 g mol^{-1}), phylloquinone (450.70 g mol^{-1}), and 17 α -ethynylestradiol (296.40 g mol^{-1}) are shown (taken and adapted from Boeren *et al.*).^[18] The molecules shown had a concentration of 400 μM (400 pmol / g ice, or 56 pmol mm^{-2}), which allowed a better understanding of their detectability with ORIGIN. Similar to the PAH study, each lipid investigated showed its own unique fragmentation pattern with its parent peak and a few minor fragment peaks. While for 17 α -ethynylestradiol and α -tocopherol the parent peak is the most abundant signal in the recorded spectrum (see Fig. 7), a much lower intensity was observed for

phyloquinone, having the most intense peak at $[M-2]^+$. Concentration scans conducted in the range of 7 fmol mm^{-2} to 28 pmol mm^{-2} on the three presented lipids, allowed the derivation of the theoretical limit of detection (at the 3 sigma level, $\text{LOD}_{3\sigma}$). The $\text{LOD}_{3\sigma}$ values for α -tocopherol, phyloquinone, and 17α -ethynylestradiol of 34 fmol mm^{-2} , 85 fmol mm^{-2} , and 0.2 fmol mm^{-2} , respectively, were derived from this campaign ($< 100 \text{ fmol mm}^{-2}$, 710 fmol / g ice). In Boeren *et al.* we also demonstrated the reliable detection and identification of mixtures of the three classes of organic molecules studied so far, namely amino acids, PAHs, and lipids (see Fig. 4 in said publication),^[18] which represents a real milestone in the application of this detection technology.

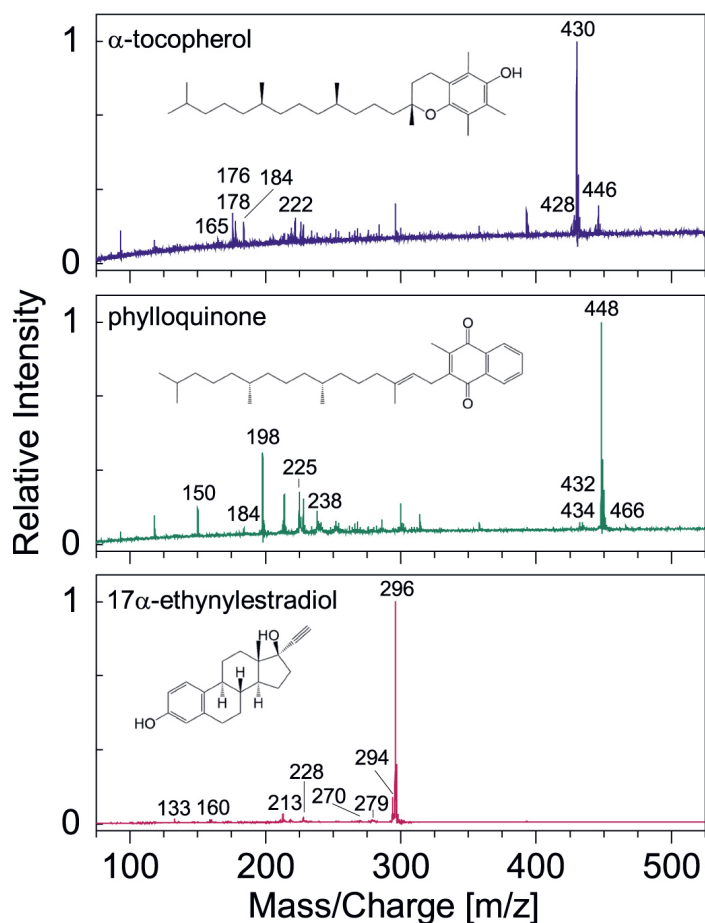


Fig. 7. Laser desorption mass spectra of α -tocopherol, phyloquinone, and 17α -ethynylestradiol standards are shown. The standards examined had a concentration of $400 \mu\text{M}$. Image taken and adapted from Boeren *et al.* ref. [18].

3.3 Nucleobases

More recently, the identical measurement protocol was used for the successful detection and identification of nucleobases with ORIGIN, investigating single nucleobases and mixtures of several nucleobases, at various concentrations.^[19] In Fig. 8, laser desorption mass spectra of adenine (135.1 g mol^{-1}), guanine (151.1 g mol^{-1}), uracil (112.1 g mol^{-1}), thymine (126.1 g mol^{-1}), cytosine (111.1 g mol^{-1}), and 5-methylcytosine hydrochloride (161.6 g mol^{-1}) are shown. Minimal fragmentation can be observed and in almost all cases the parent or protonated parent peak was detected. The more stable aromatic ring structure of the studied compounds, in comparison to a typical amino acid, is one reason why a limited fragmentation is observed. Interestingly, significantly more signal, thus better ionisation was observed for 5-methylcytosine than for cytosine, despite the difference of only one methyl group.

This study also showed that increasing the number of sample surface positions from a nominal 40 to several hundred increases the detection sensitivity of the instrument by a decade (see Fig. 4 in the same publication). For example, an $\text{LOD}_{3\sigma}$ of 50 fmol mm^{-2} ($\sim 350 \text{ fmol / g ice}$) was derived for adenine, which is well in line with the mission requirements for ExoMars with the detection of $\leq 1 \text{ nmol}$ with signal-to-noise ratio (SNR) ≥ 10 ^[25] corresponding to 141 fmol mm^{-2} , or the Europa Lander mission study with 1 pmol / g ice corresponding to 141 fmol mm^{-2} .^[12] Also of note is the clean background compared to previous studies. The introduction of argon sputtering of the sample holder prior to drop casting of the analyte significantly reduced the carry-over from previous studies, allowing improved detection and a better understanding of the signatures of the molecules studied.^[19] Note, argon ion sputtering cannot and will be not applied during a space mission; it allows the re-use of sample holders during laboratory tests.

3.4 Matrix Effects and Identification Strategies

Laser desorption measurements have been conducted so far on organic compounds mixed with NaCl ,^[17] and on KCl ,^[17] $(\text{NH}_4)_2\text{SO}_4$, MgSO_4 , and CaCO_3 .^[26] Typically, only the cation of each matrix (*e.g.* Na^+) was detected as the system is operated in the positive ion mode. These mass lines typically do not interfere with the mass lines for organic compound identification. A slight interference with Ca^+ is observed with the pattern of the amino acid alanine (compare Fig. 1 in Ligterink *et al.*^[17] with Fig. 4 in Ligterink *et al.*^[26]). In the presence of NaCl slightly higher pulse energies were required to reliably detect the amino acids. The salt crust might reduce the desorption and ionization efficiency of the amino acids present in the salt/organics mixture.^[17]

For the identification of molecular structures that interfere with each other, an increase in pulse energy might provide further insights into the compounds present. The increased pulse energy results in increased fragmentation, whereas the energy thresholds at which this occurs, are compound specific. As a consequence, the newly generated fragments might allow a better insight into the molecules present.

4. Future Applications

The measurement protocol using ORIGIN is currently based on the availability of an organic residue film on a preferably conductive surface, to support the laser desorption ionisation process. Extraction of organics from soil or mineral matrices using laser ablation conditions (elevated laser irradiances at the level of GW/cm^2 or higher) would severely fragment the organic structures and consequently limit their detection. Therefore, for the application of the current measurement protocol, we are either dependent on a solvent-based organic extraction unit or the organics are in a liquid phase, such as ice or droplets, which can be collected. After receiving or collecting the material, the liquid phase may evaporate through the application of heat and/or vacuum conditions, leaving a residual film of organics. Due to the versatile measurement capabilities and high detection sensitivity of our space prototype mass spectrometer for the detection of organics, several international partners have expressed interest in using the instrument on future space exploration missions targeting different objects in the Solar System, including missions to Venus and Mars.

We are actively participating in the Morning Star Mission program, which aims to find signatures of life in the Venusian atmosphere.^[27–29] The presence of life in the atmosphere, which might act as a life-supporting habitat, could explain some observations that cannot be explained otherwise. The middle and lower cloud deck, at about 50 to 60 km above the surface, has milder temperature conditions of about 60°C and about 1 bar of pressure compared to the harsh environment at the surface (hundreds of Celsius and tens of bars).^[28] The current mission scenario foresees using

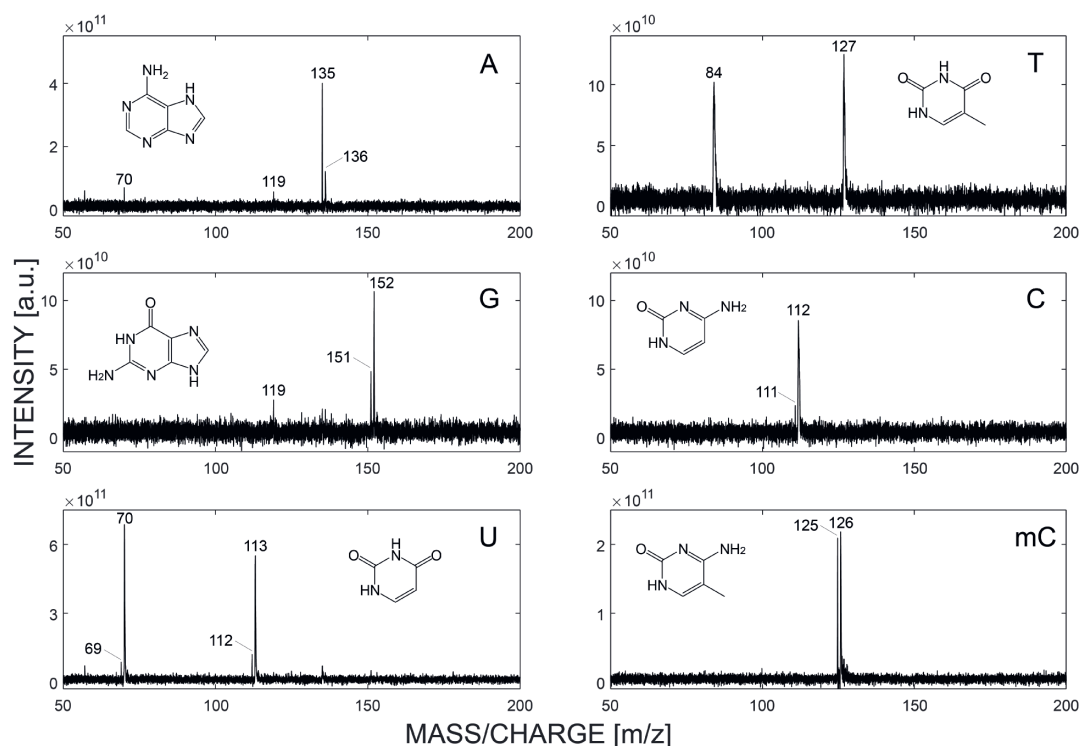


Fig. 8. Laser desorption mass spectra of adenine (A), guanine (G), uracil (U), thymine (T), cytosine (C), and 5-methylcytosine (mC) using ORIGIN. Concentrations: 5 pmol mm⁻² for A, 14 pmol mm⁻² for G, C, and mC, 707 pmol mm⁻² for U, and 141 pmol mm⁻² for T. Image adapted from Boeren *et al.* ref. [19].

a flight version of ORIGIN, where droplets will be collected in the wet atmosphere using a sampling system to be designed. Subsequently, the media in which the organics are expected, mainly sulfuric acid, will be removed by thermal heating and application of vacuum conditions (vacuum desiccation). The remaining film with possible organic material will then be analysed by ORIGIN using the measurement protocol described above.

Because of its history, Mars is one of the most promising candidates in our Solar System that still might host life or contains signatures of past life in the Martian subsurface. Together with colleagues from NASA Ames Research Center, we are working on the Abzu lander mission to Mars, that focuses on the detection of lipid signatures. NASA Ames has developed and further validated an extraction unit, named Extractor for Chemical Analysis of Lipid Biomarkers in Regolith (ExCALiBR).^[30,31] ExCALiBR accepts soil material and by solvent extraction provides a concentrated lipid extract. In Abzu,^[30] both ExCALiBR and ORIGIN are integrated into a sample carousel containing a number of sample cavities. The extract from ExCALiBR is drop cast onto a sample cavity within the sample carousel, which is then evacuated to rough vacuum conditions. The rough vacuum conditions allow the used solvents to be removed, and turning the sample cavity towards ORIGIN allows the extract to be analysed.

The icy moons Europa and Enceladus, moons of Jupiter and Saturn respectively, are other promising candidates in our Solar System^[32] where ORIGIN could find its application. The current scientific community strongly believes that the liquid oceans represent habitats where life could have flourished and been sustained. Through cracks in the ice sheets, existing life or its signatures can escape from the oceans^[33] and form deposits on the ice crust, which can be studied much more easily with a landed mission than directly in the ocean. The Europa Lander Study Report^[12] and the Enceladus Orbilander Mission Concept^[13] outline the mission objectives and for example, the required detection sensitivity of future payloads for the detection of organics. The current measurement capabilities of ORIGIN meet several mission requirements, such as detection limits or the detection of different molecule classes or molecules of such a class.^[12,17]

5. Conclusions

The detection and identification of signatures of life, past or present, on a planetary body is one of the major goals of current space research and planetary exploration. A conclusive detection of signatures of life would have a tremendous impact on science and the general public, as we would know for the first time that life is not unique to Earth. In this contribution, we have demonstrated the current measurement capabilities of our space prototype mass spectrometry system ORIGIN – a laser-based ionisation mass spectrometer operated in the desorption mode. The system has recently been developed for the detection and identification of organics related to life. To date, the gentle desorption without matrix application allows the identification of amino acids, PAHs, lipids, and nucleobases using the same measurement protocol. The measurement methodology is based on the spot-wise chemical analysis of a residual organic film and the acquisition of mass spectra for each laser shot applied. Through molecule specific fragments or the detection of the parent peak, reliable identification of the organic species is possible, even within a mixture. The current measurement versatility, together with the high detection sensitivity, allows the payload requirements to be met, for example for a landed mission on Europa or Enceladus. The system can therefore be of real added value for future space exploration missions dedicated to the detection of life.

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Author Contributions

AR, NJB, and PKS were involved in conceptualization, formal analysis, investigation, methodology, and validation. AR was involved in supervision, funding acquisition, and writing the original draft. PKS was involved in software activities. PW was involved in supervision and funding acquisition. All were involved in reviewing and editing of the manuscript.

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