

Decadal Survey on Life and Physical Sciences Research in Space 2023–2032

Topical: In situ chemical investigation of biological systems with laser-based Mass Spectrometry for the Artemis mission

Peter Wurz, Physics Institute, University of Bern, 3012 Bern, Switzerland
e-mail: peter.wurz@unibe.ch, Tel. +41 31 684 44 26

Andreas Riedo, Physics Institute, University of Bern, Sidlerstrasse 5, 3012 Bern, Switzerland

Niels F.W. Ligterink, Physics Institute, University of Bern, Sidlerstrasse 5, 3012 Bern,
Switzerland

Valentine Grimaudo, Physics Institute, University of Bern, Sidlerstrasse 5, 3012 Bern,
Switzerland

Marek Tulej, Physics Institute, University of Bern, Sidlerstrasse 5, 3012 Bern, Switzerland

Topical: In situ chemical investigation of biological systems with laser-based Mass Spectrometry for the Artemis mission

Peter Wurz, Andreas Riedo, Niels F.W. Ligterink, Valentine Grimaudo, Marek Tulej
Physics Institute, University of Bern, Sidlerstrasse 5, 3012 Bern, Switzerland

In the framework of the Artemis mission a range of biological experiments on the lunar surface are foreseen, e.g., the lunar environment biology campaign, to study the impact of realistic space environment (low gravity, temperate, vacuum) on model organisms. To study these biological systems, their growth, survival, and decay, we propose to employ laser-based mass spectrometry to investigate the chemicals involved in the metabolism, organic molecules related to living matter, and specific biological compounds, of the organisms used in these studies in detail, and ideally in real-time.

Introduction

In the framework of the Artemis mission a range of biological experiments on the lunar surface are foreseen, e.g., the lunar environment biology campaign [1], which will allow to study the impact of realistic space environment (low gravity, temperate, vacuum) on e.g., model organisms. A large group of the necessary analytics to characterize biological systems is the detailed investigation of the chemistry, and the chemical processes, in these biological samples. The chemicals involved in the metabolism, organic molecules related to living matter, and specific biological compounds, of the organisms used in these studies have to be investigated and characterized at the molecular level in great detail, and ideally in real-time. Also for the search for signatures of life on the surface of Europa identification and chemical analysis of biological relevant molecules is necessary [21]. In the laboratory, the analytical techniques of choice for such investigations are typically gas-chromatography mass spectrometry (GC-MS), liquid chromatography (LC, LC-MS), high-performance liquid chromatography (HPLC, HPLC-MS), which are large and complex instruments, therefore not very suitable in the context for space exploration where resources are very limited, and the instrumentation should not be complex.

In recent years, laser-based mass spectrometry for space exploration advanced significantly to become a promising alternative for these measurements [2, 3, 4, 5, 6]. Many biologically relevant compounds can easily and simply be determined with laser-based mass spectrometry, allowing for a continuous monitoring and analysis of the biological experiments exposed to real space conditions.

The Laser-based Mass Spectrometer

Laser-based mass spectrometric (LIMS) measurements were conducted using our prototype mass spectrometric system built at the University of Bern, Switzerland. The system was originally designed for the *in situ* chemical analysis of solids (elements and

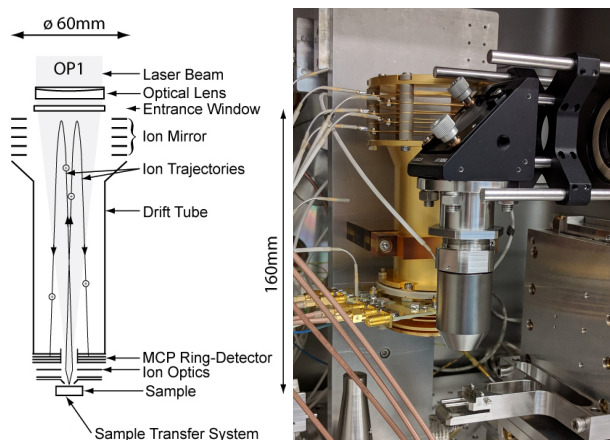


Fig. 1: LIMS instrument: Left schematic drawing of the LIMS instrument; Right: the actual mass spectrometer (golden structure in the back) and the microscopic imaging system in front [18]. The sample is moved between the two by a translation stage with μm resolution and repeatability.

isotopes) on planetary bodies [2]. Detailed information about the design and principles of operation can be found in previous publications [see e.g., 7, 8, 9, 10, 11, 6, 12, 13, 14]. In the following only a brief description of measurement principles is given. The schematics of the current measurement set-up and LIMS principles of operation are illustrated in Fig. 1. The LIMS system consists of a miniature reflectron-type time-of-flight mass spectrometer (RTOF, analyzer with geometrical dimensions of 160 mm x Ø 60 mm, installed within vacuum chamber with typical base pressure of mid 10^{-8} mbar) which is coupled to a laser system for removal and ionization of sample material. The laser beam is guided through the mass analyzer towards the sample surface and focused to small spot sizes. The present performance of LIMS provides a mass resolution $m/\Delta m$ of up to about 1100, full mass spectrometric analysis over a wide mass range of 1 – 1000 u, an accuracy of the mass scale of 500 ppm, and a dynamic range of 8 decades. The mass range of a TOF instrument is theoretically unlimited, but practically limited by the data acquisition time and the memory size. However, there are no system level constraints for the mass range, such as RF amplitude and frequency (quadrupoles and quistors) or magnetic field strength (sector instruments).

Operating LIMS in desorption mode (with low laser intensities) allows for the gentle desorption of chemical compounds, which is used for the detection of molecules and biomolecules present on the investigated surface [1, 15]. Low laser irradiances on the sample cause the desorption of neutral and ionized molecules and volatile atoms of the sample [16]. The positive ions generated during the laser desorption and ionization process immediately enter the mass analyzer to maximize the detection efficiency. The ions are accelerated, confined, and focused to the field-free drift path. At the ion mirror (reflectron), the ions are reflected towards the detector system and pass the drift path a second time. We use a nanosecond pulsed Q-switched Nd:YAG laser ($\lambda = 266$ nm, pulse duration ~ 3 ns, pulse repetition rate of 20 Hz) providing a focal spot size of about 30 μm diameter. TOF spectra are recorded with a high-speed digitizer system, and an in-house written software-suite was used for the data analysis that includes e.g., signal integration, conversion of TOF to mass spectrum. The sample holder is positioned directly below the mass analyzer on a three-dimensional translation stage with micrometer position accuracy.

Molecular and biomolecular samples are prepared in solutions at nM to μM levels that are drop-casted into small cavities of the sample holder at about μL volumes [17]. After evaporation of the solvent, the samples are introduced into the vacuum system for analysis with the LIMS instrument. LIMS measurements provide full mass spectrometric analysis over a mass range of 1 – 1000 u, all within seconds, enabling detection of biomolecules at surface concentrations down to fmol mm^{-2} .

In flight, these biomolecule extracts would be provided directly from a sample biological system (e.g., 2-D cultures, 3-D cultures, Tissue-on-a-Chip, and multi-physiological systems) or from a dedicated solvent extraction instrument to provide concentrated extractions, e.g., lipids, from the various biological experiments. For example, ExCALiBR [19] performs solvent extraction of target lipids (fatty acids, alkanes, and polycyclic aromatic hydrocarbons) and purification from a soil sample for subsequent molecular characterization by analytical instruments, from e.g., experiments performed on lunar regolith.

Examples of bio-relevant molecules detected with LIMS

In the context of biology, the mass spectrometric detection and characterization of a range of organic molecules, amino acids, lipids (fatty acids, vitamins, sterols, glycerides, phospholipids, and others), and more, will allow us to investigate the state of the organisms of the biological experiments on the lunar surface [20]. With LIMS, such compounds can be investigated from

extracts produced from organic matter, or directly from biofilms on the test surfaces of the biological experiments. Moreover, compounds resulting from the metabolic process, chemicals from the environment that have adverse effects (biocide) on the growth of microorganisms can be easily detected with LIMS. In the following we give a few examples of the detection of complex biomolecules.

Fig. 2 shows LIMS measurements in desorption mode of biotic and abiotic amino acids [15]. For example, one requirement in the Europa Lander Science Definition report [21] for the detection of signatures of life is the identification of eight of the following amino acids: Ala, Asp, Glu, His, Leu, Ser, Val, Iva, Gly, β -Ala, GABA, and AIB. We successfully measured ten of these amino acids with our LIMS instrument (β -Ala and Iva were not included in our study).

The mass spectra of the amino acids are simple to interpret, they show the parent mass peak and a few high-mass fragment peaks. This unique and simple pattern identified for each amino acid can be used to decompose the mass spectra and identify all amino acids and their relative abundance in a mix of amino acids with certainty. This also allows to distinguish between proteinogenic amino acids and abiotic amino acids, the latter possibly originating from meteoritic infall onto the lunar surface, considering that chondritic meteorites contribute about 2 wt% of material to the lunar regolith [22, 23]. With our LIMS instrument operating in desorption mode we established the 3σ limits of detection ($LOD_{3\sigma}$) for amino acids, which are at a surface concentration between 1 and 600 fmol mm⁻², depending on the amino acid [15]. Moreover, these amino acids were also detected in the presence of NaCl and KCl salts in the sample, as shown in Fig. 2 [15]. A mixture of 12 amino acids was successfully analyzed with a LIMS instrument using an orbitrap mass analyzer at surface concentrations of about 1 pmol/mm² [28].

Lipids are important biomolecules [25], their functions include storing energy, signaling, and acting as structural components of cell membranes. Lipids are known to be affected by UV and particle radiation, resulting e.g. oxidation, possibly resulting in cell death.

Fig. 3 shows two mass spectra of prenyl lipids, with intensities normalized to the most intense peak, where 200 pmol vitamin K₁ (top) and α -tocopherol (bottom) were drop-casted for analysis. The most intense mass peak in the spectra was identified as the parent peak for both lipids, vitamin K₁ and α -tocopherol (see labels). The molecular structures of both lipids are shown in their corresponding mass spectra. The grey boxes in both mass spectra denote

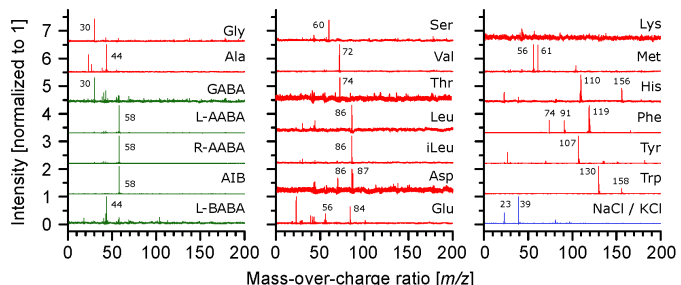


Fig. 2: LIMS mass spectra in desorption mode of various biotic (red) and abiotic (green) amino acids, and of a mixture of NaCl / KCl salt (blue) [15]. The average surface concentration is 14 pmol mm⁻² for the pure amino acids, and ~0.7 μ g mm⁻² for the mixture with NaCl / KCl salt. The primary fragments are labelled with their mass.

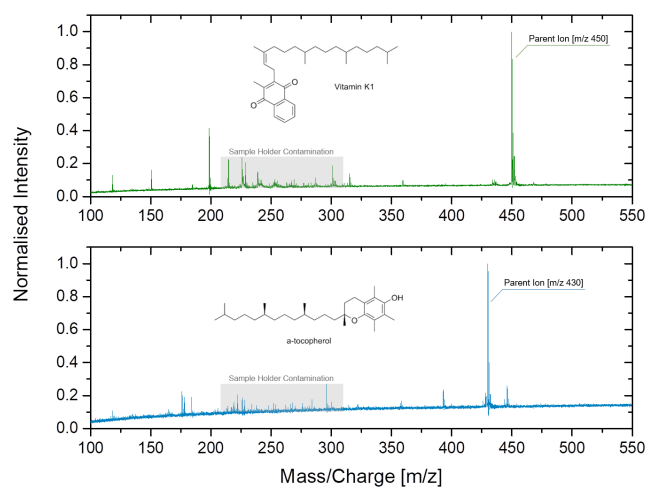


Fig. 3: Mass spectra of two lipids recorded using our LIMS system operated in desorption mode [26]. The top panel shows Phylloquinone (vitamin K₁) and the bottom panel shows A-tocopherol (vitamin E).

the contribution of sample holder contamination. Several other lipids were successfully detected with our LIMS instrument in desorption mode as well [26], which were menadione (vitamin K3), retinol (vitamin A1), cholecalciferol (vitamin D3), and ethinyl-estradiol. Similarly, phthalic acid in a clay sample (Mars regolith simulant) has been detected in the presence of perchlorates with a LIMS instrument [24].

Polycyclic aromatic hydrocarbons (PAH) are complex chemical compounds containing only carbon and hydrogen that are organized in structures of multiple aromatic rings. In nature, PAHs can be formed from organic sediments, but PAHs are also part of chondritic meteorites [27], and are a prominent component in inter- and circumstellar material. Fig. 4 shows several measurements of PAHs recorded with our LIMS instrument [17], as an example of large organic molecules, where again the mass spectra of these PAHs are simple to interpret, they show mostly the parent mass peak and a few high-mass fragment peaks. Although PAHs are not synthesized directly by biological processes but are degraded by certain types of bacteria, PAHs are also of interest, because of their potential role in the emergence of life and their abundance in the interstellar medium. Similar results on pyrene have been obtained with a laser-desorption laser-ionization mass spectrometer [6].

Conclusion

Modern LIMS instruments, which couple pulsed lasers with time-of-flight mass spectrometers, are highly capable, compact, and adaptable mass spectrometers for a wide range of analytical questions. With LIMS operating in a mode optimized for the detection and characterization of chemical compounds, we have a simple, robust, instrument for analyzing complex chemical compounds to support the biological research studies foreseen on the lunar surface in the framework of the Artemis mission [1]. LIMS could be used e.g., to directly investigate and monitor the biological samples in long-term experiments, or in combination with other instruments for specific extracts derived from the biological experiments, to understand the impact of the lunar environment on biology.

In the fundamental biology research studies enabled by the Artemis III mission the identification and characterization of underlying mechanisms affecting biological matter play an important role in discovering the key physiological processes that are altered by the lunar environment and aid in identifying biomarkers for diagnostics and specific targets for countermeasures, and understanding countermeasure effects, specific and side-effects.

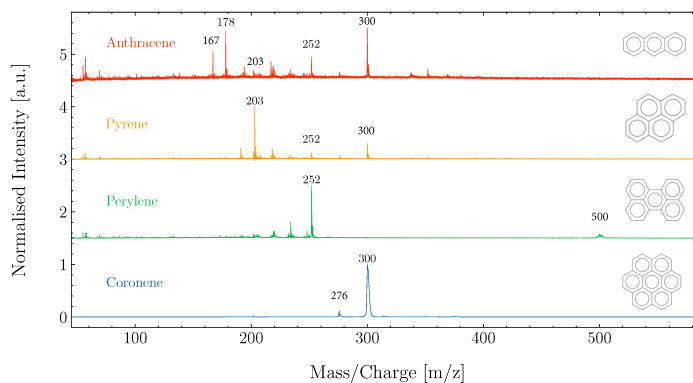


Fig. 4: Polycyclic aromatic hydrocarbons (PAH) are chemical compounds containing only carbon and hydrogen that are composed of multiple aromatic rings. LIMS mass spectra recorded in desorption mode of four PAH species [17]. From bottom to top, the PAHs displayed are coronene (blue), perylene (green), pyrene (orange), and anthracene (red). A concentration of 100 μM has been used for all PAHs in this measurement. Prominent peaks are indicated in the plot. The spectra have been normalized to the highest peak for the respective PAH and have been offset from each other. The chemical structure of the PAHs is shown on the right side.

References

- [1] Artemis III, Science Definition Report, NASA/SP-20205009602
- [2] P. Wurz, P. Tulej, R. Lukmanov, V. Grimaudo, S. Gruchola, K. Kipfer, C. de Koning, N. Boeren, L. Schwander, R. Keresztes Schmidt, N.F.W. Ligterink, and A. Riedo, Identifying biosignatures on Planetary Surfaces with Laser-based Mass Spectrometry, IEEE Aerospace Conference Big Sky, MT, USA, (2022), submitted.
- [3] P. Wurz, M. Tulej, A. Riedo, V. Grimaudo, R. Lukmanov, and N. Thomas, Investigation of the Surface Composition by Laser Ablation/Ionisation Mass Spectrometry, IEEE Aerospace Conference Big Sky, MT, USA, (2021), 50100, 15 pages, DOI: 10.1109/AERO50100.2021.9438486.
- [4] F. Goesmann, W.B. Brinckerhoff, F. Raulin, W. Goetz, R.M. Danell, S.A. Getty, S. Siljeström, H. Mißbach, H. Steininger, R.D. Arevalo Jr., A. Buch, C. Freissinet, A. Grubisic, U.J. Meierhenrich, V.T. Pinnick, F. Stalport, C. Szopa, J.L. Vago, R. Lindner, M.D. Schulte, J.R. Brucato, D.P. Glavin, N. Grand, X. Li, and F.H. W. van Amerom; the MOMA Science Team, The Mars Organic Molecule Analyzer (MOMA) Instrument: Characterization of Organic Material in Martian Sediments, *Astrobiology* 17(6/7), (2017) 655–685, DOI: 10.1089/ast.2016.1551.
- [5] W. Goetz, W.B. Brinckerhoff, R. Arevalo Jr., C. Freissinet, S. Getty, D.P. Glavin, S. Siljeström, A. Buch, F. Stalport, A. Grubisic, X. Li, V. Pinnick, R. Danell, F.H.W. van Amerom, F. Goesmann, H. Steininger, N. Grand, F. Raulin, C. Szopa, U. Meierhenrich, J.R. Brucato and the MOMA Science Team, MOMA: the challenge to search for organics and biosignatures on Mars, *International Journal of Astrobiology* 15(3), (2016), 239-250, DOI: 10.1017/S1473550416000227.
- [6] S. A. Getty, W. B. Brinckerhoff, T. Cornish, S. Ecelberger and M. Floyd, Compact two-step laser time-of-flight mass spectrometer for in situ analyses of aromatic organics on planetary missions, *Rapid Communications in Mass Spectrometry* 26, (2012), 2786–2790, DOI:10.1002/rcm.6393.
- [7] V. Grimaudo, P. Moreno-García, A. Riedo, M.B. Neuland, M. Tulej, P. Broekmann, and P. Wurz, High-Resolution Chemical Depth Profiling of Solid Material Using a Miniature Laser Ablation/Ionization Mass Spectrometer, *Analytical Chemistry*, 87, (2015), 2037–2041, DOI: 10.1021/ac504403j.
- [8] V. Grimaudo, P. Moreno-García, A. Riedo, S. Meyer, M. Tulej, M.B. Neuland, M. Mohos, C. Gütz, S.R. Waldvogel, P. Wurz, and P. Broekmann, Toward Three-Dimensional Chemical Imaging of Ternary Cu–Sn–Pb Alloys Using Femtosecond Laser Ablation/Ionization Mass Spectrometry, *Analytical Chemistry*, 89, (2017), 1632–1641, DOI: 10.1021/acs.analchem.6b03738.
- [9] A. Riedo A., Bieler A., Neuland M., Tulej M., and Wurz P., Performance evaluation of a miniature laser ablation time-of-flight mass spectrometer designed for in situ investigations in planetary space research, *Journal of Mass Spectrometry*, 48, (2013), 1–15, DOI: 10.1002/jms.3157.
- [10] A. Riedo, C. d. Koning, A.H. Stevens, C.S. Cockell, A. McDonald, A. Cedeño López, V. Grimaudo, M. Tulej, P. Wurz, and P. Ehrenfreund (2020) The detection of elemental signatures of microbes in Martian mudstone analogues using high-spatial resolution laser ablation ionization mass spectrometry. *Astrobiology*, 20(10), (2020), 12 pages, DOI: 10.1089/ast.2019.2087.
- [11] P. Wurz, M. Tulej, A. Riedo, V. Grimaudo, R. Lukmanov, and N. Thomas, Investigation of the Surface Composition by Laser Ablation/Ionisation Mass Spectrometry, IEEE Aerospace Conference Big Sky, MT, USA, (2021), 50100, 15 pages, DOI: 10.1109/AERO50100.2021.9438486.

- [12] A. Riedo, M. Neuland, S. Meyer, M. Tulej, and P. Wurz, Coupling of LMS with a fs-laser ablation ion source: elemental and isotope composition measurements, *Journal of Analytical Atomic Spectrometry*, 28, (2013), 1256–1269, DOI: 10.1039/C3JA50117E.
- [13] M. Tulej, N.F.W. Ligterink, C. de Koning, V. Grimaudo, R. Lukmanov, P. Keresztes Schmidt, A. Riedo, and P. Wurz, Current Progress in Femtosecond Laser Ablation/Ionisation Time-of-Flight Mass Spectrometry, *Applied Sciences*, 11, (2021), 2562, DOI: 10.3390/app11062562.
- [14] M. Tulej, A. Neubeck, M. Ivarsson, A. Riedo, M.B. Neuland, S. Meyer, and P. Wurz, Chemical Composition of Micrometer-Sized Filaments in an Aragonite Host by a Miniature Laser Ablation/Ionization Mass Spectrometer, *Astrobiology*, 15, (2015) 669–682, DOI: 10.1089/ast.2015.1304.
- [15] N.F.W. Ligterink, V. Grimaudo, P. Moreno-García, R. Lukmanov, M. Tulej, I. Leya, R. Lindner, P. Wurz, C.S. Cockell, P. Ehrenfreund, and A. Riedo, ORIGIN: a novel and compact Laser Desorption - Mass Spectrometry system for sensitive in situ detection of amino acids on extraterrestrial surfaces, *Nature Science Reports* 10:9641, (2020), 10 pages, DOI: 10.1038/s41598-020-66240-1.
- [16] P. Wurz, K.R. Lykke, M.J. Pellin, D.M. Gruen, D.H. Parker, Characterization of Fullerenes by Laser-Based Mass Spectrometry, *Vacuum* 43 (1992), 381–385, DOI: 10.1016/0042-207X(92)90041-T.
- [17] K. Kipfer, N.F.W. Ligterink, J. Bouwman, L. Schwander, V. Grimaudo, C. de Koning, N. Boeren, P. Keresztes Schmidt, R. Lukmanov, M. Tulej, P. Wurz, and A. Riedo, Towards Detecting Carbon Chemistry on Planetary Objects with the ORIGIN Space Instrument, *Planet. Sci. Jou.* (2021), submitted.
- [18] R. Wiesendanger, V. Grimaudo, P. Moreno, A. Cedeño López, A. Riedo, M. Tulej, A. Neubeck, M. Ivarsson, D. Wacey, H. Shea and P. Wurz, Chemical and optical identification of micrometre sized 1.9 billion-year-old fossils by combining a miniature LIMS system with an optical microscope, *Astrobiology*, 18(8), (2018), 1071-1080, DOI: 10.1089/ast.2017.1780.
- [19] M.B. Wilhelm, A.J. Ricco, M. Chin, J.L. Eigenbrode, L. Jahnke, P.M. Furlong, D.K. Buckner, T. Chinn, K. Sridhar, T. McClure, T. Boone, L. Radosevich, A. Rademacher, T. Hoac, M. Anderson, S. Getty, A. Southard, R. Williams, X. Li, T. Smith, O. Podlaha, and J. van Winden, ExCALiBR: An Instrument for Uncovering the Origin of the Moon’s Organics, *Lunar Surface Science Workshop*, (2020), Abstract id. 2241
- [20] P. Schwendner, A. Riedo, D.J. Melton, P. Horvath, R. Lindner, P. Ehrenfreund, K. Beblo-Vranesevic, P. Rettberg, E. Rabbow, F. Westall, C. Moissl-Eichinger, L. Garcia-Descalzo, F. Gomez, R. Amils, V. Þór Marteinsson, N. Walter, and C.S. Cockell, Biological degradation of amino acids as a potential biosignature, *Frontiers in Astrobiology*, (2021), submitted.
- [21] K.P. Hand, A.E. Murray, J.B. Garvin, W.B. Brinckerhoff, B.C. Christiner, K.S. Edgett, B.L. Ehlmann, C.R. German, A.G. Hays, T.M. Hoehler, S.M. Hörst, J.I. Lunine, K.H. Nealson, C. Paranicas, B.E. Schmidt, D.E. Smith, A.R. Rhoden, M.J. Russel, A.S. Templeton, P.A. Willis, R.A. Yingst, C.B. Phillips, M.L. Cable, K.L. Craft, A.E. Hofmann, T.A. Nordheim, R.P. Pappalardo, and P.E. Team, (2017) Report of the European Lander Science Definition Team.
- [22] J.J. Papike, G. Ryder, C.K. Shearer, Lunar samples, *Rev. Mineral.* 36 (5), (1998), 1–234, DOI: 10.1515/9781501508806-020.
- [23] *Lunar Sourcebook*, ed. Grant H. Heiken, David T. Vaniman, Bevan M. French, 1991, Cambridge University Press, USA

- [24] S. A. Getty, W. B. Brinckerhoff, R. D. Arevalo, M. M. Floyd, X. Li, T.A. Cornish, and S. Ecelberger, Miniature Laser Desorption/Ionization Time-of-Flight Mass Spectrometer for in Situ Analysis of Mars Surface Composition and Identification of Hazard in Advance of Future Manned Exploration, proceedings of the Concepts and Approaches to Mars Exploration Meeting, Houston, TX, USA, 2021
- [25] J. W. Aerts, W.F.M. Röling, A. Elsaesser, and P. Ehrenfreund, Biota and Biomolecules in Extreme Environments on Earth: Implications for Life Detection on Mars, *Life*, 4, (2014), 535–565; DOI:10.3390/life4040535
- [26] N. Boeren, V. Grimaudo, S. Gruchola, C. de Koning, P. Keresztes Schmidt, K. Kipfer, N.F.W. Ligterink, R. Lukmanov, M. Tulej, P. Wurz, and A. Riedo, Detecting lipids on planetary surfaces with laser-based mass spectrometry, *Planet. Sci. Jou.* (2021), submitted.
- [27] M.R. Wing and J.L. Bada, Geochromatography on the parent body of the carbonaceous chondrite Ivuna, *Geochim. Cosmochim. Acta*, 55(10), 2937–2942, DOI: 10.1016/0016-7037(91)90458-H.
- [28] R. Arevalo Jr., L. Selliez, C. Briois, N. Carrasco, L. Thirkell, B. Cherville, F. Colin, B. Gaubicher, B. Farcy, X. Li, and A. Makarov, An Orbitrap-based laser desorption/ablation mass spectrometer designed for spaceflight, *Rapid Commun Mass Spectrom.*, 32 (2018), 1875–1886, DOI: 10.1002/rcm.8244.