Detection of biomarkers using compact and sensitive laser ablation/desorption ionization mass spectrometry.
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Introduction: The in situ detection of biomarkers on Solar system bodies is extremely challenging. Because of the limited resources available on a lander or rover platform (e.g., power, mass, and volume), the commonly used laboratory systems in life sciences cannot be applied. Therefore, to conclusively answer the question if life has existed or still does beyond our Earth, novel and sensitive measurement techniques are required for future space exploration missions to e.g., Mars or the icy moon Europa. Hays et al. [1] reviewed in detail various biosignatures and grouped them into six categories, ranging from biomolecules (amino acids, lipids, etc.) to microscopic structures such as micrometer-sized fossils. From this review it is clear that a subset of high-performing and complementary instrumentation is required to detect signatures of life reliably. In our contribution, we present the current measurement capabilities of our sensitive Laser Ablation/Desorption Ionization Mass Spectrometers (LIMS). LIMS operated in ablation mode allows amongst other capabilities, the detection of a single microbe (of interest to e.g., Mars exploration), whereas LIMS operated in desorption regime enables the detection of amino acids with concentration levels down to the femtomol per mm^2 (Europa as potential target). The LIMS instrument can be coupled to a miniature microscope camera system that extends the measurement capabilities to context analysis of solids, which is of high importance with regard to the analysis of heterogeneous solid materials.

Laser Ablation/Desorption Ionization Mass Spectrometry: The two discussed LIMS setups consist of the same miniature reflectron-type time-of-flight (RTOF) mass analyzer (160 mm x Ø 60 mm, see Fig. 1). The analyzer is coupled to either a femtosecond laser system (laser pulse with of ~190 fs, 1 kHz pulse repletion rate, wavelengths from 775 nm down to 258 nm using a THG) for ablation studies of solids, or to a nanosecond laser system (pulse width of ~3 ns, 20 Hz, wavelength of 266 nm) for desorption studies of molecules. In both mass spectrometric setups, the mass analyzer is located within a vacuum chamber that is evacuated down to the mid 10^{-8} mbar level. The laser system is currently installed outside the vacuum chamber with a beam guiding system for beam delivery to the sample. The laser beam is focused by a lens system through the mass analyzer, along its central axis, towards the sample (spot sizes from ~10–30 µm), which is installed just below the entrance of the mass analyzer, in close vicinity (~1 mm) to the entrance of the ion optical system. During material ablation/desorption, positively charged ions are generated that are guided towards the field-free drift path of the TOF analyzer. At the ion mirror the direction of travel of the ions is reversed, thereby passing the drift tube a second time and hitting the detector system. Ions of different mass-to-charge ratios arrive sequentially at the detector system (TOF principle). High-speed measurement cards are used to record the ion signal. The detection system is designed in such way that for each applied laser shot, a full mass spectrum is recorded.

Fig. 1 Miniature mass analyzer (in gold) and high-resolution microscope system (in grey). The mass analyzer has a dimension of 160 mm x Ø 60 mm. Combined, they enable chemical context analysis of solids.

A high-resolution camera system is coupled to the LIMS system that allows precise targeting of the sample material with the laser beam (see Fig. 1). The camera system has a resolution of 1 µm.

Sample material: In this contribution, three different measurement campaigns of high interest to the detection of biosignatures will be discussed in more detail. The investigated material includes i) Martian mudstone analogues artificially inoculated with microbes (~10^6 cells / cm^3) [2-3], ii) drop-casted solutions of single amino acids standards, or mixtures thereof, with concentrations at the µM level [4], and iii)

a geological sample containing fossil structures of micrometer dimensions [5, 6].

Measurement Results and Discussion: The chemical depth profiling measurement methodology established earlier using LIMS [7] allowed successful spatially resolved chemical analysis of solids with a lateral resolution of about 10 µm (limited by the ablation spot size) and a vertical resolution at the nanometer level. This methodology allowed the localization of fossil veins in an aragonite host by monitoring bio-relevant elements, and hence its chemical composition without significant contribution from the host material [5]. Moreover, very recently the same methodology allowed the identification and the localization of single microbes artificially inoculated in Martian mudstone analogues by post-screening the recorded data for carbon layers [2-3]. The elemental analysis of these carbon layers allowed for the differentiation between abiotic and biotic carbon layers, which is of high importance in such studies. In biotic cases, a simultaneous increase of the CHNOPS elements was observed while in abiotic cases an increase of carbon was solely detected [2]. Note that only spatially resolved chemical analysis at the spatial scale of the microbes themselves enabled the identification of these microbes. No signatures were observed when using bulk measurement techniques for these samples, since the microbes were sparsely distributed [3]. Since many biosignatures are at micrometer dimensions, the contribution from the host material might completely mask the faint signal from these biosignatures when using bulk techniques. Therefore, spatially resolved chemical analysis at micrometer level and below is of high importance in astrobiology.

Laser desorption studies with a nanosecond laser system were recently conducted on single amino acid standards and mixtures thereof. The investigated solutions had concentration levels down to µM and were drop-casted (1 µL) into cavities (0.2 mm x Ø 3 mm) on a steel sample holder at ambient conditions. Before analysis, the solvent was let to evaporate, resulting in a thin film of biomolecules inside the cavities. Spot-wise rastering (typically 40 position, on each position 100 laser shots) allowed for the identification of simple and amino acid unique fragmentation patterns. These patterns were used for the successful identification of amino acids in complex mixtures. With the current measurement methodology, amino acids at concentration levels down to the femtomol per mm^2 can be identified.

Currently, a laboratory-scale nanosecond laser is used for biomolecule detection. Such systems can be replaced by sophisticated and robust pulsed microchip laser systems. In Figure 3, such a microchip laser system is shown – note the dimensions of only 100 mm x 34 mm - and is currently being validated for the application with our miniature mass analyzer. The model shown in Fig. 3 outputs a wavelength of 532 nm, provides up to 40 µJ per laser pulse (which is sufficient for our desorption studies), and can be operated at a pulse repetition rate of up to 200 Hz. The pulse energy jitter is below 1%, which is comparable to laboratory-scale femtosecond laser systems. In the near future three different types of such microchip lasers, each one operating at a different wavelength (1064 nm, 532 nm, 266 nm), will be tested for biomolecule desorption studies.

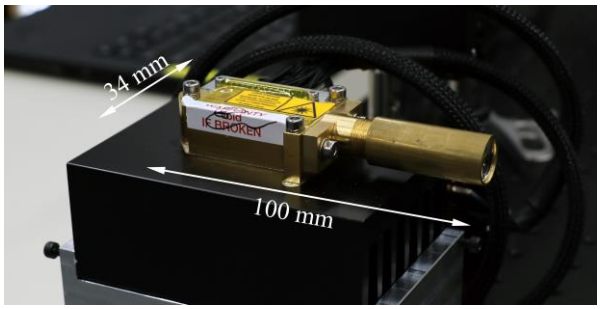

Fig. 2 Pulsed microchip laser system suitable for coupling to the miniature mass analyzer for, e.g., remote molecular desorption studies.

Conclusion: Measurement campaigns conducted on various astrobiologically relevant materials demonstrate that LIMS operated in ablation/desorption regime is a measurement technique suitable for the detection of an important subset of signatures of life. The high detection sensitivity of the miniature mass analyzer coupled with the capability of providing spatially resolved chemical analysis allows for the localization and identification of biologically relevant structures, such as fossils or single microbes, as well as the sensitive identification of biomolecules such as amino acids at concentration levels down to femtomol per mm^2. These figures of merit are of special interest for future space exploration missions devoted for life detection, such as a potential Europa Lander Mission from NASA.

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References: [1] Hays L.E. et al. (2017) *Astrobiol., 17*, 363–400. [2] Riedo A. et al. (2020) *Astrobiol., 20*, 1224–1235. [3] Stevens A.H. et al. (2019) *Nature Sci. Rep., 9*, 9706. [4] Ligterink N.F.W. et al. (2020) *Nature Sci. Rep., 10*, 9641. [5] Tulej M. et al. (2015) *Astrobiol., 15*, 669–682. [6] Tulej M. et al. (2020) *J. Mass. Spectrom., 55*, e4660. [7] Grimaudo V. et al. (2015) *Anal. Chem., 87*, 2037–2041.