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A New Method and Mass-Spectrometric Instrument for Extraterrestrial Microbial Life Detection Using the Elemental Composition Analyses of Martian Regolith and Permafrost/Ice

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Abstract

We propose a new technique for the detection of microorganisms by elemental composition analyses of a sample extracted from regolith, permafrost, and ice of extraterrestrial bodies. We also describe the design of the ABIMAS instrument, which consists of the onboard time-of-flight laser mass-reflectron (TOF LMR) and the sample preparation unit (SPU) for biomass extraction. This instrument was initially approved to fly on board the ExoMars 2020 lander mission. The instrument can be used to analyze the elemental composition of possible extraterrestrial microbial communities and compare it to that of terrestrial microorganisms.

We have conducted numerous laboratory studies to confirm the possibility of biomass identification via the following biomarkers: P/S and Ca/K ratios, and C and N abundances. We underline that only the combination of these factors will allow one to discriminate microbial samples from geological ones.

Our technique has been tested experimentally in numerous laboratory trials on cultures of microorganisms and polar permafrost samples as terrestrial analogues for martian polar soils.

We discuss various methods of extracting microorganisms and sample preparation. The developed technique can be used to search for and identify microorganisms in different martian samples and in the subsurface of other planets, satellites, comets, and asteroids—in particular, Europa, Ganymede, and Enceladus. Key Words: Mass spectrometry—Life-detection instruments—Biomarkers—Earth Mars—Biomass spectra. Astrobiology 17, 448–458.

1. Introduction

The problem of determining the origin of life in the Universe is one of the most interesting, popular, and hardest to solve in modern science (Abyzov *et al.,* 2006; Duxbury *et al.,* 2006; Botta *et al.,* 2008). However, it is still not known when, in what environment, and under what conditions living matter emerged, self-replication was established, and transfer of the properties from one generation to subsequent ones occurred. In this regard, the search, localization, and identification of life-forms beyond Earth can significantly advance our understanding of how life appeared on our planet. It has been shown that conditions on Europa (Managadze, 2009; Managadze *et al.* 2009) and on Mars (Managadze *et al.,* 2012) could be conducive for the emergence and evolution of living matter.

The ability to deliver landers and rovers to the martian surface, with a significant scientific payload, has allowed scientists to proceed to the first stage of the martian habitability assessment (Clark *et al.,* 1976; Abyzov *et al.,* 2006; Grotzinger *et al.,* 2014). The results of these studies have shown that 2–3 billion years ago Mars had all the necessary conditions for the emergence and evolution of microorganisms. As is the case for terrestrial microorganisms, martian microbes might have existed at considerable depths below the surface (Duxbury *et al.,* 2001, 2004; Farr *et al.,*

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2002; Managadze, 2013). Moreover, even after the loss of atmosphere and water on Mars, these microbial communities could have survived and persisted in deep layers of rocks (Pedersen, 1993; Stevens and McKinley, 1995), sediments (Gilichinsky *et al.,* 1992, 2007; Vorobyova *et al.,* 1997; Gal'chenko, 2004; Lever *et al.,* 2013; Lever and Teske, 2015), and ices (Abyzov, 1993; Miteva *et al.,* 2004; Managadze, 2009; Managadze *et al.,* 2009; Knowlton *et al.,* 2013). The question about the duration of survival and possible replication of a potential martian biomass remains open. However, answers with regard to the occurrence of a martian biota are attainable, given the available data on the recovery of DNA from ancient terrestrial permafrost (Johnson *et al.,* 2007). In addition, it has been shown experimentally that sublimation of ice within permafrost, in the presence of a temperature gradient, and diffusion of water vapor through the regolith, can lead to the formation of a ''wet layer'' with a water content of up to 30 wt %. This is sufficient to maintain metabolism and growth of microorganisms at positive and even subzero temperatures (Pavlov *et al.,* 2010).

These advances have stimulated the development of new research areas, in particular, experimental exobiology and techniques of extraterrestrial life detection. In unmanned missions, the scientific payload on landers and rovers is limited. This has encouraged the development of robust methods and instruments that are focused on specific biological analysis, for example, biological chips, and also multifunctional equipment to investigate biomarkers as well as physicochemical or mineralogical parameters of the environment.

Mars is a prime target for astrobiological research using increasingly diverse and complex scientific payloads and techniques. Among the spectral techniques implemented to search for potential biomarkers in the martian regolith are Raman, infrared, and fluorescence spectrometry; gas chromatography–mass spectrometry (GC-MS); mass spectrometry of ionized neutral atoms (INMS); laser-induced breakdown spectroscopy (LIBS); and matrix-assisted laser desorption ionization (MALDI) (Korablev *et al.,* 2010; Bottger *et al.,* 2012; Wurz *et al.,* 2012; Bishop *et al.,* 2013; Bower *et al.,* 2013; Edwards *et al.,* 2013; Getty *et al.,* 2013). However, some of these techniques have limitations such as low sensitivity to certain elements or complexity of data interpretation. In our instrument, the sample is placed into the vacuum chamber, and the pressure of the residual gas (orders of magnitude lower than the atmospheric pressure on Mars) eliminates the influence of the ambient gas.

Developed for the Phobos-Grunt mission, a laser ablation time-of-flight mass spectrometer (LA TOF MS) LASMA is able to solve multiple tasks in the study of regolith composition and structure. A comprehensive next-generation device based on LASMA has been developed (Managadze, 1992; Managadze *et al.,* 2010). Combining laser ablation with the soft ionization technique, this device allows for study of the following:

(1) elemental and isotopic composition of samples by laser ablation and ionization;
(2) molecular ions of samples in soft ionization mode.

Using this equipment, we have proposed and developed novel techniques and a new generation of instruments for the search for extraterrestrial microorganisms. Samples can be acquired from sediments, dust, ice (Managadze, 2009; Managadze *et al.,* 2009, 2012), and atmospheric aerosols.

The main goal of the present study was to describe a novel technique and onboard LA TOF MS (with a microorganism extraction system) for extraterrestrial life detection. In this case, the special task was to ensure the identification of biological matter by measuring the elemental composition of samples isolated from their geological environment.

Our method and instrumentation have significant advantages over approaches that attempt to identify extraterrestrial biological matter by detecting complex organic compounds. These methods are primarily attributed to such onboard instruments as MALDI and gas chromatograph–mass spectrometer. The latter can detect the breaking of organic substances mirror symmetry in the regolith and ice matrix. The advantage of our method, as will be shown below, is the lack of complex sample preparation. For instance, the sample preparation for MALDI may require many manipulations including centrifugation and mixing with the matrix.

Our results show that the proposed procedure can be performed quickly and reliably by an onboard sample preparation system. We have mitigated additional difficulties associated with the highly complex preliminary procedures in registering organic compounds for a MALDI-class instrument.

Also, our LA TOF MS requires little or no additional configuration changes after the in-house and post-landing commissioning. A laboratory prototype of the proposed instrument's predecessor was developed in 1985; thus 30 years of operational knowledge exists.

A laboratory prototype of the spaceborne instrument based on LASMA TOF MS after significant improvements was used to detect the microbial biomass in soil and ice/permafrost samples via analysis of the elemental composition. For this analysis, we chose as biomarkers P/S and Ca/K ratios, and abundances of N and C. Our approach is based on the selectivity of nutrient absorption by microbial cells from the environment. Previously, it was found that the ratios P/S and Ca/K inside microorganisms change over the life cycle and depend on the physiological state of the cells. Also, fossilized microorganisms can be distinguished from microorganisms present in the active state (Mulyukin *et al.,* 2002). The X-ray microanalysis makes the detection of cells in a native mineral environment possible due to the selective accumulation of these elements by cells (Sorokin *et al.,* 2003).

Here, we propose and describe a technique that uses laser ablation time-of-flight mass spectrometry elemental analysis of a sample for the detection of microbial biomass in native samples (soil, permafrost, ice).

Based on these results, this instrument—ABIMAS—was initially selected for the ExoMars science payload. An important reason for the selection was that the proposed instrument also can perform high-precision measurements of the elemental and isotopic composition of martian regolith and rocks.

ABIMAS, designed to be placed on the lander, consists of two parts: a compact upgraded laser mass spectrometer LASMA in a vacuum chamber equipped with a pumping system, and a special sample preparation unit (SPU) for processing biological samples. The samples in ABIMAS can

be loaded via an onboard manipulator. The method can be applied to microorganisms that may be in an icy shallow subsurface of Mars or the jovian moons, especially Europa. The instrument can also provide information about the chemical composition of dust that settles from the atmosphere onto the instrument sample holder.

2. Materials and Methods

2.1. LA TOF MS to measure the elemental composition of samples

The spectrometer (Fig. 1) has an axial symmetry relative to both laser emission and the motion of the plasma ions of the plume. This configuration ensures high reproducibility of the mass spectra and the capability to analyze the sample layers down to a depth of 1–2 mm.

After preparation, the sample is placed into the vacuum chamber (not shown on the scheme) via the rotating disk (RD) with a set of holes. After supplying the sample, the vacuum chamber is sealed by compression of the seal ring. The reference sample (permanently installed on the disk) is used to check the regular functioning of the device before operation. To ensure functioning in the martian atmosphere, the ion optics (REF, T, OPT), the detector (DET), and the sample (S) are placed in a cylindrical vacuum chamber with windows. These windows serve to input the laser beam, electrical connectors for power switching for the analyzer and the ion detector, the output signal from the detector, as well as a flange for joining a pumping system. The operating pressure in the vacuum chamber is maintained via a compact turbomolecular pump.

The laser radiation reaches the top of the sample by passing through the mesh of the reflector with a transparency up to 80% and through the central hole in the detector. The irradiation of a sample is performed by the optical node of the device that includes a Nd:YAG laser (LASER) with a wavelength of 1.06 μm, the optical system consisting of a focusing lens, electro-optical attenuator (ATT), and the control unit for the laser pulse energy measurement and synchronization. The Nd:YAG laser with passive Q-switching provides an output energy of the laser pulse of 20 mJ; its duration of $\sim$7 ns and the maximum repetition rate can reach 1 Hz. The laser can operate in a wide temperature range from −50°C to +50°C. The laser radiation is focused on the target surface as a spot with a diameter of 30–90 μm and power density of $\sim 10^9$ W/cm^2. The attenuator allows for changing the energy of the laser pulse through a range of 40 dB in small increments. This is required to precisely adjust the laser ion source for singly charged ions, as well as to compensate for the change in the energy of the laser emitter under the influence of temperature. This allows for selection of an arbitrary mode of laser operation, providing a wide range in the degree of ionization and the energy of produced ions. The spot movement on the target surface is controlled by deflecting the laser beam. The positioning accuracy of the spot on the target plane is about 50 μm.

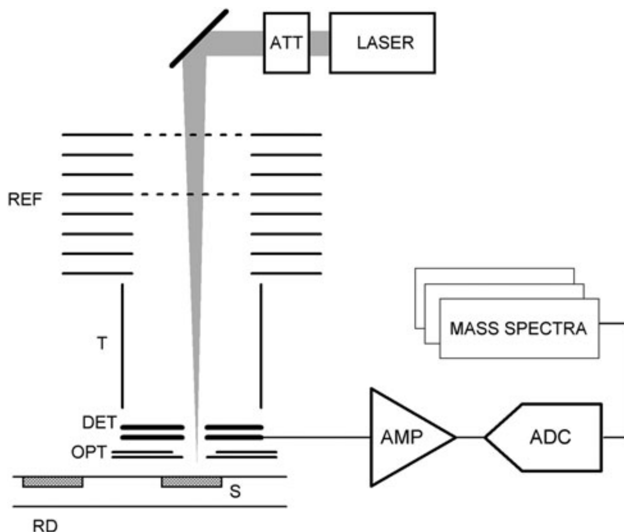

FIG. 1. A simplified scheme of the mass spectrometer. LASER = laser module; ATT = electro-optical attenuator; AMP = amplifier; ADC = analog-to-digital convertor; REF = ion reflector; T = drift tube; DET = ion detector; OPT = ion optics; S = sample under investigation; RD = rotating disk acting as a sample holder.

The laser pulse causes ablation, complete dissociation, and ionization of sample material within the focal spot. Its intense heating ensures the emission of the target material in the form of plasma ions. High-velocity ions emitted from the target (in a free expansion regime) are confined by the ion optics (OPT) and guided into the drift tube (T), where they are separated by time of flight proportional to their mass. After reflection in a retarding electrostatic field of the ion reflector (REF), the ions are recorded by the secondary electron multiplier—a microchannel plate assembly (DET). The output signal from the electron multiplier is fed through the amplifier (AMP) to the high-speed analog-to-digital converter (ADC) and stored in memory as a single spectrum.

From the time of flight of the ions and the intensity of their mass peaks, elemental and isotopic composition of the substance under investigation can be accurately determined. The most important feature of the device is that the laser ion source, during a single laser shot, allows almost stoichiometric output of any element from hydrogen to uranium and, therefore, allows their registration with the high throughput of the analyzer (Managadze and Managadze, 1997).

The sensitivity of the mass spectrometer is at least 50 ppmA in a single spectrum and 5 ppmA in a histogram of 100 spectra. It grows as a square root of N, the increase in the number of spectra added to the array. The typical accuracy for determining the abundance of elements (with a concentration in a sample of about 0.1%) is 0.005% in 500 spectra. And it is less than 0.002% for elements with a concentration of about 1%. Even better accuracy can be achieved by increasing the number of measured spectra.

The program that processes mass spectra arrays can present these results in the form of tables and performs fast, automatic comparisons of the values of biomarkers, biologically important elements, and micro admixtures. The presence of biomass in the sample can be determined with a probability of error better than 1%, which is an order of magnitude higher than the probability of error when considering only the ratios Ca/K and P/S.

The electronics module provides complete control of ABIMAS, recording, storage, and transfer of spectral data

and telemetry, and it provides power to all units of the device, including the SPU. There is an option to perform initial spectra selection to reduce data transfer to Earth. The weight of the mass spectrometer unit is about 4.5 kg. The weight of the SPU is about 0.5–0.6 kg, making the total weight of ABIMAS ~5.0–5.1 kg.

2.2. SPU and biological experiments

The laboratory prototype of the SPU (Fig. 2) was developed, manufactured, and successfully tested in the Laboratory of Active Diagnostics and Mass Spectrometry, Space Research Institute, Russian Academy of Sciences. The unit was designed as a vertical cylinder with a height of 5 cm and a volume of 10 cm^3. This volume is limited on both sides by sealed rotary valves (rotary shutters, RV1, RV2). The top shutter is for the input of the regolith with a volume of 1–2 cm^3. The bottom shutter is used to dispose the sample residue after the extraction of microorganisms from the regolith and discharge water after washing the volume. The maximum water amount that can be held in the currently used liquid tank (LT) is about 100 cm^3. For each sample preparation and subsequent cleaning procedure, the SPU uses approximately 10–15 cm^3 of water. Thus, the SPU can process up to six samples with cleaning of the volume between each run. The limitation of the total number of processed samples depends on how much water can be transported to Mars. If the mission profile allows for an increase of the mass limit, it is possible to increase the volume of the liquid container and, accordingly, the number of analyzed samples.

It is known that Mars' regolith has a number of water-soluble salts that contain K and Ca (Toner *et al.,* 2014). In high-salinity environments, the elements in the mass spectrum can have elevated concentrations that are identical to elements of biomarkers. This can prevent correct measurements; hence there is the need to purify the sample from the water-soluble salts. Desalination of polar soils under laboratory conditions was carried out with miniature cartridges (C1, C2) that are included in the sample preparation laboratory prototype device.

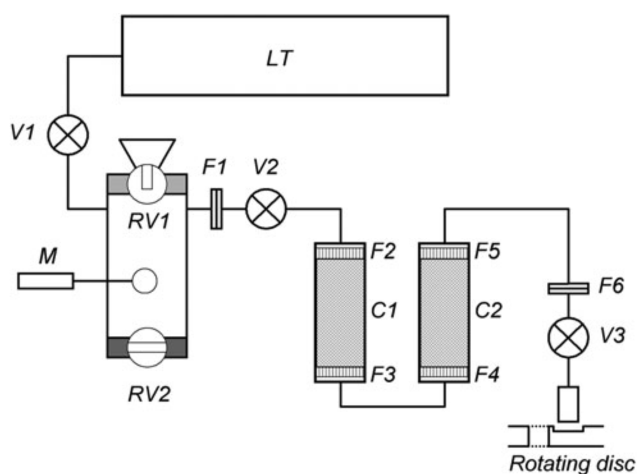

FIG. 2. A scheme of the SPU. V1–V3 = valves; F1–F6 = filters; RV1, RV2 = rotary valves; C1, C2 = ion exchange cartridges; LT = liquid tank; M = motorized mixer.

The results of our experiments with the samples of ancient permafrost are described in Fig. 3.

There is also a need, after the deposition of the coarse fractions and extraction of the microorganisms from the regolith (before the input of the new sample material), to remove particles and salts from the volume.

The method consists of the following procedures: after loading 1–2 cm^3 of powdered soil sample into the SPU, the volume is filled with 5–6 mL of sterile pyrogen-free water. Then the suspension is mixed to break up soil conglomerates. During this mixing, a partial separation of microorganisms and heavy mineral particles occurs.

According to the rough estimate based on Stoke's law, the sedimentation velocity of mineral particles in Mars' weak gravitational field is 30–50% slower; thus the process takes 2–3 times longer compared to terrestrial conditions. We experimentally determined that the characteristic time for the solid fraction deposition of the mixture is about 5 min. Under Mars' gravity, the estimated time will be 10–15 min. After the deposition of the suspension, the top layer is discharged into cartridges (C1, C2) that contain a mixed layer of ion-exchange anion resin AV-17 and cation resin KU-2. Their total action achieves a water purification factor of 10^2 to 10^3, given the initial 1500 ppm. After purification, the suspension, rich in microorganisms, is supplied to the target's substrate. The water evaporation from the surface of the metal substrate ensures the formation of the layer of microorganisms and microparticles. Subsequently, we put the target for the analysis into the mass spectrometer on the focal plane of the laser beam.

The control of liquid flow is performed by the valves V1–V3. Also, there are several filters (F1–F6) that prevent clogging of the SPU system.

3. Results

To investigate the potential of the proposed method and instrumentation, we performed biological experiments with soil and permafrost samples that contain different volumes of microbial biomass. It is known that unfrozen water in terrestrial permafrost contains high concentrations of salts. The salt concentration increases in unfrozen water upon freezing, which lowers the freezing temperature.

Thus, long-existing microorganisms in ancient terrestrial permafrost are hypersaline-dwelling extremophiles.

According to our previous research, many bacterial isolates from ancient polar permafrost can grow in a liquid medium with 12–20% NaCl (meaning they were halotolerant). Others exhibit weak growth at high salinity but preserve their viability for a significant period of time at 20% NaCl in the growth medium (Soina and Vorobyova, 2004). However, after thawing of the ancient Arctic and Antarctic permafrost samples and the addition of salt-free water, large-scale cellular death (as a result of lysis of cells adapted to salts) did not occur.

Transmission electron microscopic (TEM) studies of bacterial cells from ancient permafrost, which were released after thawing and concentrated after centrifugal water washing procedures (Bae *et al.,* 1972), did not show any signs of cellular rupture following the immersion into a hypotonic environment. Most cells were observed as small forms (0.2–0.5 μm), single or in conglomerates, surrounded

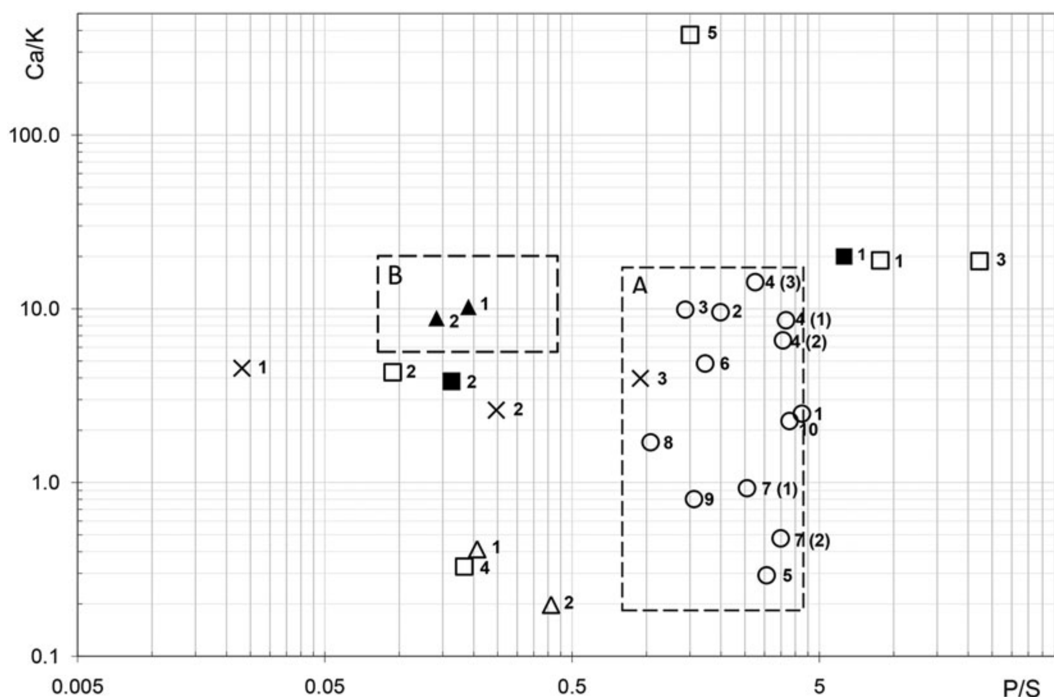

FIG. 3. Ca/K versus P/S plot. Microorganisms: ○1 = *Arthrobacter castelli;* ○2 = *Arthrobacter polychromogenes;* ○3 = *Arthrobacter polychromogenes;* ○4 (1) = *Arthrobacter roseus;* ○4 (2) = *Arthrobacter roseus;* ○4 (3) = *Arthrobacter roseus;* ○5 = *Bacillus cereus;* ○6 = *Kocuria rosea;* ○7 (1) = *Micrococcus luteus;* ○7 (2) = *Micrococcus luteus;* ○8 = *Mycobacterium* sp.; ○9 = *Pseudomonas stutzeri;* ○10 = *Saccharomyces cerevisiae.*

Permafrost samples processed by the SPU: △1 = Frozen sediments, Tiksi, depth 8.8 m; △2 = Frozen sediments, Tiksi, depth 2.75 m; ×1 = sterile frozen sediments, Tiksi, depth 8.8 m; ×2 = frozen sediments, 2 days at 30°C; ×3 = frozen sediments, 2 months at 25°C.

Martian regolith composition: ▲1 = Mars Pathfinder, Sojourner's APXS, 1997; ▲2 = JSC Mars-1 martian regolith model.

Geostandards, USGS certificates: □1 = Basalt Hawaiian Volcano Observatory, BHVO-2; □2 = Green River Shale, SGR-1; □3 = Basalt Hawaiian Volcano Observatory, BHVO-1; □4 = Marine Sediment, MAG-1; □5 = Icelandic Basalt, BIR-1.

Geological standards, according to the instrument measurements: ■1 = Basalt Hawaiian Volcano Observatory, BHVO-2; ■2 = Green River Shale, SGR-1.

A = approximate area corresponding to microorganisms; B = expected values for the martian regolith without microorganisms.

by surface capsular layers covered with organomineral particles. Bacterial cells *in situ* exhibited undamaged cell walls, nucleoids, and cytoplasm. The capsular surface layers, in contrast to isolates from unfrozen soils and subsoil sediments, remained after numerous passages of bacterial isolates. Other cells (0.8–1 μm) also revealed capsular layers, thickened cell walls, and an electron-dense cytoplasm, resembling dormant forms like cysts. Some cells were L-shaped and lacked cell walls. These data correlate with biodiversity and high numbers of bacteria isolated from water suspensions of permafrost samples as well as with high total numbers of viable cells (10^7 to 10^8 cells/gwt) counted directly by epifluorescence microscopic technique.

Moreover, bacterial isolates from permafrost (Gr+ and Gr-) preserved viability after 30 cycles of freezing (–20°C) and thawing (20°C) in water suspensions, and 80–87% of cells remained culturable. TEM studies did not reveal any visible lesions in cell ultrastructure. Cells were characterized by a high density of the cytoplasm and stable cell wall structure (Soina and Vorobyova, 2004; Soina *et al.,* 2004).

Below, we describe an experiment with the Arctic permafrost sample (depth 8.8 m, southeast of the Lena River harbor), which was divided into three parts. One part was heated for 3 h at 600°C and used as the sterile control sample. In the unheated sample, the succession of microbial communities was initiated by the thawing process during two different periods of incubation: 2 days and 2 months at 25°C. The sterile control sample was similarly moistened by sterile water and incubated for 2 months at 25°C. After incubation, 1 mL/g of sterile water was added to all samples. The suspension was shaken with a vortex mixer and then centrifuged at 3000*g* for 5 min. From the supernatant layer, which contained desorbed cells and fine mineral particles, aliquots were taken for mass spectrometry analysis. Simultaneously, aliquots were taken for a direct microscopic total cell count (DAPI). After 2 days of incubation, the total cell numbers increased from an average of 10^8 cell/gdw to 10^{11} cell/gdw. The mass spectra indicate a clear shift in P/S (varied from 0.02 to 0.9) along with an increase in the microbial biomass *in situ.*

In addition to permafrost samples that contain native biomass, experiments with atmospheric aerosol samples and pure bacterial cultures immobilized in sterile soil or montmorillonite were carried out. The following cultures of bacteria isolated from Arctic and Antarctic permafrost and arid soils were studied: *Arthrobacter polychromogenes,*

Arthrobacter castelli, Arthrobacter parietis, Pseudomonas stutzeri, Kocuria rosea, Micrococcus luteus, Bacillus sp. We conducted numerous laboratory studies to confirm the possibility of biomass identification via the main biogenic element ratios P/S and Ca/K, and C and N abundances. We emphasize that only the combination of these factors will help discriminate microbial samples from geological ones. We demonstrated that all elements of interest were recorded with high mass resolution and accuracy. The results of these measurements and the area of experimental data dispersion are shown in Fig. 3. The obtained data allow us to define the ''area of life'' corresponding to the samples containing microorganisms of at least 1000 cells per gram.

We also investigated geological rock standards (Basalt BHVO, Columbia River Basalt BCR-2), as well as the data for known martian rocks (McLennan *et al.,* 2014). These data, as a rule, do not fall into the area of the chart that corresponds to the microorganisms.

The Icelandic Basalt BIR-1, Green River Shale SGR-1, and the Basalt Hawaiian Volcano Observatory samples BHVO-2 and BHVO-1 are shown in Fig. 3, according to the data from the US Geological Survey (USGS) certificates. Out of those samples, we measured with our instrument the following two: the Basalt Hawaiian Volcano Observatory, BHVO-2, and the Green River Shale, SGR-1. Our results are close to the USGS reference certificate data.

The Icelandic Basalt BIR-1 exhibits an extraordinarily high Ca/K ratio because it is poorer in K (K_2O—0.03 wt %) than is BHVO-2 (K_2O—0.52 wt %), while the Ca content is about the same in both samples (CaO—13.3 wt % in BIR-1 and 11.4 wt % in BHVO-2).

The Green River Shale SGR-1 as well as the Basalt Hawaiian Volcano Observatory samples BHVO-2 and BHVO-1 show very high P/S ratios. These levels are higher than the levels in microbial samples. It helps distinguish them from the microbial cultures and from the samples of permafrost enriched in viable microorganisms, which we studied.

Due to the peculiarity of the LA TOF MS relative sensitivity coefficients, we were able to determine adjacent mass peak ratios, for example, for K and Ca. The calibration of the proposed method was conducted by using the soil model, which had about 10^6 cells per $1\,cm^3$ of the sample. Using 50 averaged spectra as a comparison standard, we obtained spectra in which the mass peaks of biomarkers had signal-to-noise ratios greater than 3×10^3. This sensitivity allows for reliable detection of microorganisms when their concentration in a sample is $10^3\ cm^{-3}$. However, this value is not a limit.

The sensitivity may be increased 6 times by using a simple, passive optical element—a laser beam splitter. This element mounted within the laser radiation beam creates six separate focal spots instead of one, which are arranged in a circle on the target. Another possibility of increasing the sensitivity is to increase the repetition rate or the number of laser pulses.

Thus, if this value is increased from 1 to 30 Hz, that is, 30 times, the sensitivity will increase by about 5.5 times. We have great experience working with similar emitters. Onboard diode-pumped laser emitters are similar to the lamp-pumped lasers by weight, dimensions, and power consumption; thus they can be used. However, there are some technological problems and high cost issues. The joint

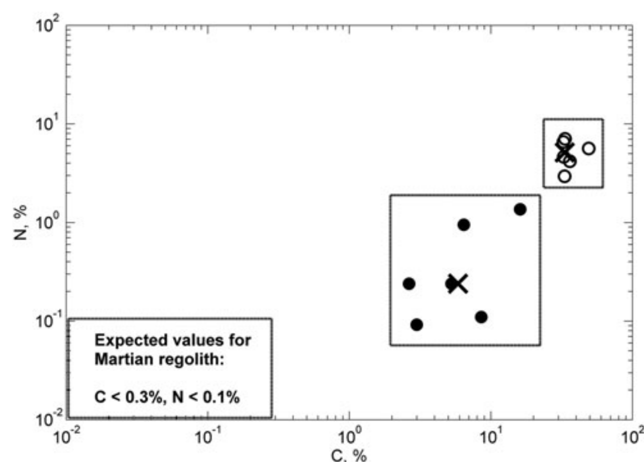

FIG. 4. Carbon and nitrogen abundances. A K-means cluster analysis for the carbon and nitrogen abundance data. The hollow circles correspond to microbial cultures; the filled circles correspond to permafrost samples processed by the SPU. The cluster centroids are marked with crosses. The L1 distance, 2 clusters, 25 centroids replications were used as parameters for clustering.

utilization of all the possibilities can provide 2 orders of magnitude increase in the instrument's sensitivity. This enables the detection of microorganisms with a concentration of $10\,cm^{-3}$, which is a sufficiently high sensitivity for a compact onboard instrument.

Our method is based on the fact that the matrix structure of any terrestrial biological substance contains about the same amount of elements H, N, C, O. It was essential that the ratios of these elements in living organisms could be accurately reproduced. Moreover, due to their high concentration in terrestrial organisms, they can be easily detected.

We note that, of all the matrix elements, the content of N and C can be the most reliably reconstructed. The content of O depends on the degree of dehydration of the living biomass, while the content of N and C is not affected by the humidity.

For microorganisms, the N and C content values significantly exceed 1%, while the latest data show that the concentration of C and N for martian rocks does not exceed ~0.3% and ~0.1%, respectively (Stern *et al.,* 2015). Figure 4 has the values of matrix biomarkers as units on its axes and shows that their use significantly increases the reliability of living matter detection. We performed a K-means cluster analysis for the N and C chart. It has clearly separated the areas containing data from bacterial cultures and native permafrost samples processed by the SPU.

4. Discussion

Assuming that, during the first few hundred million years, Mars had a warm, wet environment similar to that of early Earth and life arose on Earth as rapidly as we think, then perhaps life arose as quickly on Mars.

The processes of microbial adaption would have begun when Mars started to gradually lose its dense atmosphere. This process has been associated with the impact of solar wind particles on the upper atmosphere. This could have

lasted for more than one hundred million years and provided the emergence of a new generation of radiation-resistant microbes. The loss of the dense atmosphere led to a gradual increase in cosmic and solar UV radiation and evaporation of water reservoirs on the entire planet. However, these changes occurred slowly, which would have allowed time for microbes to adapt under stress.

Almost all UV radiation should be effectively absorbed in the first millimeters of any exposed rock surface (Cockell and Raven, 2004). Collisions with protons from either galactic cosmic rays or solar cosmic rays (with energies >10 MeV) or any secondary cascade particles (mostly neutrons) would result in the destruction of the original organic matter and putative living cells (Pavlov *et al.,* 2012). Different particles of ionizing radiation are not equally hazardous to cells. Heavy particles (alpha particles and heavy ions) are very damaging, but they compose a relatively small part of galactic cosmic rays (10% and 0.1%, respectively) and are effectively absorbed by the atmosphere. The protons and high-charge/high-energy (HZE) ions are highly ionizing and more damaging to microorganisms (Dartnell *et al.,* 2007). According to the calculations of Pavlov *et al.* (2012), the accumulation rate decreases with depth (in ice, Fe_2O_3, ''standard'' rocks), since low-energy cosmic rays (20–100 MeV) cannot penetrate deep into the rock or ice and do not produce a significant number of secondary particles. As a result, the total maximum radiation dosage occurs at the surface.

It is known that drying and low temperatures can increase radiation resistance of microorganisms. Therefore, extant microbial spores would be able to live in shallow subsurface deposits of dust and sand on Mars. They would also be transported by winds to considerable distances during dust storms. Mass-spectrometric studies conducted by Curiosity (Hassler *et al.,* 2014) found all the chemical elements (including nitrogen) necessary for the formation of living matter in dust deposits and revealed that the conditions in sediments can be suitable for microbial life.

According to the data obtained by the RAD device on Curiosity, the martian surface radiation dose was derived to be $\sim 7 \times 10^7$ Gy during 1 billion years. The lethal dose for microorganisms similar to *Deinococcus radiodurans* is believed to equal $\sim 8 \times 10^4$ Gy (Dartnell *et al.,* 2010). Therefore, microbes like *Deinococcus radiodurans* could survive in the martian near-surface regolith and permafrost for more than $\sim$1 million years [the lower estimate for the time of acquiring the lethal dose in a case without adaptation: lethal dose/accumulation rate $\sim 8 \times 10^4$ Gy/(7×10^7Gy/10^9 years)], and this is not the limit. In our laboratory studies, microbial communities from native soils and permafrost were treated *''in situ''* with ionizing radiation with a dose of up to 10^5 Gy at a pressure of 1 mbar, a temperature of −50°C, and in the presence of 5% perchlorates. Those microbial communities were capable of metabolic and reproductive activity and kept high biodiversity (Vorobyova *et al.,* 2014; Cheptsov *et al.,* 2015). The microbes exhibit the ability to repair the genome within several hours after sustaining damage and the unique ability to adapt and survive in extreme conditions (Amato *et al.,* 2010).

The most detrimental factor for non-adapted microbes on the martian surface is direct solar UV radiation. This factor, however, may be relatively small if the dynamics of dust processes during impacts of meteorites is taken into account. Currently, about 400 small-diameter impact craters occur each year on Mars. Due to the lack of a dense atmosphere, the martian surface is affected by bodies with the diameter of a few meters. An impact by a body with a diameter of 5 m will cause an intermediate crater with an average diameter of 25 m and a depth of 15 m.

During impacts, subsurface extant microorganisms would be raised to the surface together with dust. Viable microbes would be transported to the surface from the low-temperature areas that surround the crater at the final stage of crater formation. Dust clouds would provide reliable protection from direct solar UV radiation for non-adapted microbes. The other possible mechanism of UV shielding is coverage of microorganisms with opaque layers of dust owing to buildup of iron-rich particles on the ''sticky'' outer layer of microbial cells.

According to Fedorova *et al.* (2013), the martian atmosphere contains particles with two characteristic sizes: 0.7 and 1.3 μm, with a concentration of 0.1–10 cm^{-3}, and fine particles with a size of 0.1–0.01 μm, with a concentration of 10^2 to 10^4 cm^{-3}. Their chemical composition is still unknown. Terrestrial particles with such dimensions of 0.8–1.0 and 1.2–1.5 μm can correspond to microbial spores or dormant cells. This contributes to the hypothesis that some of the particles with a diameter of 1 μm may be martian microbes or their spores. Under the influence of wind, fine particles (compared to 1 μm particles) will rise earlier and drop later, providing a protective ''shield.''

According to Landis and Jenkins (2000), during one sol in calm weather, about 0.25% of the rover's solar battery will be covered by dust with a particle size of 1–2 μm. Under the same conditions for ABIMAS, there would be $\sim$10 particles in the focal spot of the laser, with a total mass of $\sim 10^{-10}$ g. If the absolute sensitivity of the proposed instrument (for 100 spectra) is $\sim 10^{-16}$ g, the measurement accuracy will be better than 1%.

The LA TOF MS described in this work has unique capabilities and sensitivity and provides elemental composition measurements of particles $\sim$1 μm in size. It will be sufficient for the analysis to collect dust particles on the instrument holder placed in the martian atmosphere during one sol. The original method proposed in this work would allow for the identification of microorganisms in martian dust via measuring biomarkers. If the dust analysis were to suggest the presence of microorganisms, a case could be made for martian aerobiology. A continuation of this work is the search for the best landing site by using a more precise location of the martian prime meridian from integrated mission data (Duxbury *et al.,* 2014). Our instrument can also be used for the conventional solid sample elemental compositional analysis.

With regard to our method, we also investigated the issue of potential damage to microbial cells that are adapted to hypersaline environments. Terrestrial extreme halophiles thrive in liquid and moistened environments with salt concentrations that approach saturation (Zhilina and Zavarzin, 1990; Galinski and Trüper, 1994). They have also been isolated from rock salts of great geological age (evaporites), most of which are represented by halite (Fendrihan *et al.,* 2006). These microorganisms depend on a permanently high salt concentration in the environment. Similar environments

may one day be detected on Mars. Halite has been found in SNC meteorites (Gooding, 1992).

For extracting potentially viable microorganisms from such samples, it would be important to follow the saline regime in order to exclude cellular rupture following immersion into hypotonic solutions.

Another type of osmoadaptation, which is characteristic for the majority of moderately halophilic and halotolerant microorganisms, is associated with the accumulation of osmolytes and antifreeze proteins (Burg and Ferraris, 2008; Chin, 2010). This type of osmoadaptation does not assume significant genetic changes.

Independent of an adaptation mechanism, the thermodynamic effect of osmolytes on cellular structures is the same and leads not only to salt tolerance but also to the tolerance to other stress factors that influence the water activity such as freezing, heating, and drying (Sleator and Hill, 2001).

Microorganisms from natural habitats have one more important mechanism that protects cells from stress. They have the ability to change their physiological status and transform to a resting state (Vorobyova *et al.,*1996, 1997). Fendrihan *et al.* (2012) published data on the formation of resting cells by halophilic archaea, which did not lyse in water.

Hypersalinity is typical for xerophytic habitats: arid soils, polar deserts, permafrost, and ground ice. Formation of cryogenic microstructures starts from freezing of the inter-aggregate pore solution, which increases the concentration of solutes in the pores. Extreme xerophytic environments, like the Antarctic deserts (Dry Valleys), are considered to be the best terrestrial analogues for a number of environments on Mars (Wentworth *et al.* 2005; Gilichinsky *et al.,* 2007; Heldmann *et al.* 2013).

Dry permafrost, as observed in University Valley, where the ice in permafrost originates from vapor deposition rather than from liquid water, is common in the northern polar regions of Mars at the Phoenix landing site (Levy *et al.* 2009; Goordial *et al.* 2016).

Concentrations of Na, Mg, K, Ca, and Cl in the terrestrial permafrost samples and arid soil samples we investigated (Table 1) are similar to the corresponding Phoenix lander mission data for Mars (Toner *et al.,* 2014). Our method can provide information about samples that have the potential to contain microorganisms and about the overall environment as well.

Table 1. Total Ion Concentrations (in m*M*) in Samples of Arid Soils (SN, S1) and Ancient Permafrost (from East Siberian Permafrost Deposits M-1/91, 380-3/92; Antarctic Beacon Valley permafrost A-6/99-6), Which Were Used in Our Experiments

	SN	*S1*	*M-1/91*	*A-6/99-6*	*380-3/92*
NH_4^+	0.009	0.010	0.007	0.006	0.011
Cl^-	0.066	0.059	0.056	0.070	0.180
Na^+	0.892	0.518	0.152	1.592	0.174
Mn_2^+	0.981	0.117	0.002	0.241	0.021
Mg_2^+	0.227	1.201	0.284	0.017	0.033
K^+	0.900	0.386	0.042	0.110	0.010

It is possible to use a destructive technique, such as laser ablation time-of-flight mass spectrometry, after nondestructive methods, such as visual investigation and Raman spectroscopy. However, in this case an extra device would be needed to transport intact samples between the instruments. It should be noted that the total number of samples processed by the SPU depends on how much water the mission can transport to Mars. However, after depletion of the liquid tank, our instrument is still able to perform the analysis of unprocessed samples.

5. Conclusions

The aim of the present study was to experimentally confirm the validity of the proposed method and instruments in the search for microbial life beyond Earth. We have shown that this major hurdle can be overcome by using the onboard LA TOF MS in conjunction with the original system of extraction and sample preparation. The mass spectra of materials extracted from martian regolith, permafrost, and ice will allow scientists to discern whether there are indications of living matter. An important conclusion such as this would be made only after in-depth analysis of the relationship between the main biogenic element ratios P/S and Ca/K, and C and N abundances. We emphasize that only the combination of these factors will help make a distinction between samples that contain microbial biomass and those exclusively of geological origin. Such results would be compared with those obtained for terrestrial native environmental samples containing microorganisms in the laboratory with the same instrumentation. Such a comparison would help answer the question as to whether life similar to that on Earth has, at present or in the past, existed on Mars.

Our previous investigations showed that microorganisms that are adapted to high salt concentrations in permafrost easily survive many freeze-thaw cycles in water suspensions. They also survive the desorption of cells into water, preserve the high total numbers of living cells and reproductive activity without cell rupture.

Taking into account Phoenix mission data, our studies of terrestrial permafrost, and our experiments with ABIMAS, our method can be applied to the study of martian permafrost without additional protection of cells from osmotic stress.

The proposed method to identify biomass, including the LA TOF MS and the extractor of microorganisms, has been tested experimentally in numerous laboratory settings on microbial cultures and terrestrial soils and permafrost that contain microorganisms. In these trials, the analysis of results for polar permafrost samples was of utmost importance (analogues of the martian soil). The results of these tests clearly show that the design of the onboard device was legitimate and optimal. In the future, we plan to expand our sample database for natural analogues of martian regolith and rocks. One of the future tasks will be to better identify the limits of the method applicability.

The developed approach can be used to analyze samples of regolith, permafrost, and ice on Mars, as well as samples extracted from the subsurface layers of Europa, Ganymede, Enceladus, comets, and asteroids. This approach can also be used to analyze the chemical composition of martian aerosols.

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Abbreviations Used

LA TOF MS = laser ablation time-of-flight mass spectrometer
MALDI = matrix-assisted laser desorption ionization
SPU = sample preparation unit
TEM = transmission electron microscopic
USGS = US Geological Survey