Extraterrestrial Life Signature Detection Microscopy: Search and Analysis of Cells and Organics on Mars and Other Solar System Bodies

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Abstract
This paper presents a review of the space exploration for life signature search with a special focus on the fluorescence microscope we developed for the life signature search on Mars and in other sites. Considering where, what, and how to search for life signature is essential. Life signature search exploration can be performed on the Mars surface and underground, on Venus’ cloud, moon, asteroids, icy bodies (e.g., moons of Jupiter and Saturn), and so on. It is a useful strategy to consider the targeted characteristics that may be similar to those of terrestrial microorganisms, which are microorganisms with uniform spherical or rod structures with approximately 1 µm diameter surrounded by a membrane having a metabolic activity and mainly made of carbon-based molecules. These characteristics can be analyzed by using a fluorescence microscope and a combination of fluorescence pigments with specific staining characteristics to distinguish the microorganism characteristics. Section 1 introduces the space exploration for life signature search. Section 2 reviews the scientific instruments and achievements of past and ongoing Mars exploration missions closely related to astrobiology. Section 3 presents the search targets and analysis of astrobiology. Section 4 discusses the extraterrestrial life exploration methods that use a microscope together with other methods (based on mass spectrometry, morphology, detection of growth, movement, and death, etc. for microscopic and macroscopic organism). Section 5 expounds on the life signature detection fluorescence microscope, for which we have manufactured a bread board model and tested for extraterrestrial life exploration.

Keywords Extraterrestrial life · Microscope · Fluorescence · Mars · Solar system exploration · Cells · Organics · Instrument

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1 Introduction

1.1 Extraterrestrial Life Exploration

We consider extraterrestrial life exploration as one of the most important issues in space science. If extraterrestrial life were to be detected, the general conception on the universe will change, and findings will initiate a totally new biology addressing extraterrestrial life, different from the current biology addressing only the life form present on Earth. The negative result of life not being detected in a certain parameter space is also significant not only for science, but also for space utilization and planetary protection because the result will provide information on safety issues.

Different approaches can be used to detect extraterrestrial life signature. One promising approach is the performance of a high-precision characterization of exoplanets and search for the signal suggesting the presence of life, such as O$_2$, ozone, and red edge (Fujii et al. 2018). To observe these features, the difficulties caused by the extremely high contrast between the central star and the planet must be overcome. The high-precision observations of exoplanets have become an important field in the study of modern astronomy. On the contrary, the organism itself cannot be directly observed, even if the signal suggesting the presence of life is detected in extrasolar planets. Search for Extraterrestrial Intelligence (SETI) is another approach that aims to receive signals, such as radio waves, emitted by extraterrestrial civilization (Drake and Sobel 1992; Hirabayashi 2019). A huge scale radio telescope system, called the Square Kilometer Array, is going to be built including the SETI as a scientific target (Tarter 2004). However, SETI inevitably overlooks the life forms that have not evolved to intelligent civilization.

It is well known in terrestrial organisms that the evolution direction is from microorganisms to large organisms and that the number density of microorganisms is higher than that of larger organisms in our planet. It is reasonable to assume that the same is true in extraterrestrial organisms. Therefore, in this study, we mainly focus on the exploration of microorganisms and organic compound on Mars and other solar system bodies (while other targets are also discussed). In our strategy, in situ analysis is proposed to focus on searching the microorganisms and organic compound without overlooking the possible presence of these signatures (Enya et al. 2021, 2020; Saito et al. 1999; Yamagishi et al. 2016, 2010; Yoshimura 2019). It is important to note that once the possible candidate of microorganisms and organics were to be found, the samples can be returned and analyzed with the state-of-the-art technology in ground laboratory.

Organisms on Earth are well known to have evolved to adapt to their environment. This would be true for extraterrestrial life. Once it emerged, it would adapt to its extraterrestrial environments. Under the similar environment to Earth where liquid water is present and organic compound are supplied from space, it is reasonable to consider that the targeted characteristics of extraterrestrial microorganisms may be similar to those of terrestrial ones and various characteristics may be universal in any probable life in resembling environment. On the other hand, of course, it is also important to consider the possible life form different from terrestrial type without prejudice.

1.2 Mars and Other Places

Mars has been the most important site for extraterrestrial life exploration. In the 1970s, the National Aeronautics and Space Administration (NASA)’s Viking mission landed on Mars and conducted pioneering life exploration experiments. Since then, experiments related to
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astrobiology have been performed in multiple Mars missions. The studies conducted in these missions revealed the characteristics of the Martian environment billions of years ago, including the presence of large amounts of water (oceans/lakes) on its surface (Carr and Head 2003). They also found that oxygen was absent, and carbon dioxide concentrations were at least 100–1000 times higher than the present levels (Kasting 1987). Early Mars would also have a dense carbon dioxide atmosphere, a humid and warm climate (Pollack et al. 1987), and a strong magnetic field (Acuna et al. 1999). The detection of methane in the atmosphere and organic compounds in mudstone have also been reported (Eigenbrode et al. 2018; Freissinet et al. 2015; Webster et al. 2015). Studies have shown that certain life on Earth is survivable in the current Martian environment (Yamagishi et al. 2010).

Other than Mars, several places have also been cited and discussed as targets for extraterrestrial life exploration. Icy bodies, especially places with subsurface oceans and/or ejected plumes, such as Enceladus and Europa, are interesting places, as well as Venus’ atmosphere, whose habitability of the environment and possibility of life relative to phosphine detection were recently investigated (Greaves et al. 2020; Limaye et al. 2021; Porco et al. 2006).

1.3 Microscopy

For hundreds of years, optical microscopy has been one of the most important tools in biology. Robert Hooke discovered chamber-like structures in cork using an optical microscope and called them “cells” (Hooke 1665).

Organic and inorganic particles usually have little contrast under a normal optical microscope. Material-specific pigments and staining are sometimes used to enhance contrast in biological observations. Special optics (i.e., phase contrast and differential interference microscope) have also been used to enhance contrast. By utilizing fluorescent pigments in combination with a fluorescence microscope, the contrast can greatly be increased, and microorganisms and organic compounds can be detected with high sensitivity. In addition, many types of fluorescent pigments exist, each of which binds to a unique target, such as an intracellular structure or substance. The characteristics of extraterrestrial life are not obvious; hence, a combination of multiple pigments is important in understanding the target characteristics and in determining whether or not the observed target is life or not. Another feature of the analysis using a fluorescence microscope is that the shape and size of microorganisms can be observed by imaging.

With the above-mentioned features, we consider exploration using a microscope as an essential method for extraterrestrial life exploration, complementary to other important methods for detecting “materials”, e.g., mass spectrometry (MS). We have been developing a fluorescent microscope system for extraterrestrial life exploration that can be mounted on a planetary exploration spacecraft (Enya et al. 2021, 2020; Saito et al. 1999; Yamagishi et al. 2016, 2010; Yoshimura 2019).

1.4 Sample Return

The recent progress of sample-return missions for planetary exploration is remarkable. Hayabusa brought back a sample of the Itokawa asteroid (Kawaguchi et al. 1996; Tsuchiyama et al. 2011; Yoshikawa et al. 2015), while Hayabusa 2 succeeded in sampling on the Ryugu asteroid and already returned to Earth (Morota et al. 2020; Tsuda et al. 2020). The Mars Moon Exploration (MMX), a Japan Aerospace Exploration Agency (JAXA) mission to bring back samples of the Martian moon, Phobos (see Sect. 2.6 for more details), is under development (Kawakatsu et al. 2019; Usui et al. 2020). Although Fujita, Kurokawa,
and their colleagues (Fujita et al. 2019; Kurosawa et al. 2019) have assured that there is no surviving microorganisms in the samples brought back to Earth, there may be dead Martian-derived microorganisms in the Phobos regolith. The MARS2020 and Mars Sample Return (MSR) projects are also in progress (Beaty et al. 2019; Williford et al. 2018).

In situ exploration and sample-return missions are complementary in extraterrestrial life exploration. In an in situ exploration, resources that can be carried (e.g., equipment mass) are severely restricted, but there is a huge potential to acquire and examine fresh (hopefully living) samples. In contrast, the returned samples can be analyzed in detail by fully using large instruments on the ground laboratory. We believe that fluorescence microscopy is effective not only for in situ exploration, but also for the analysis of returned samples, and we consider applying it to both.

1.5 Outline of This Paper

Section 2 reviews the scientific instruments and achievements of past and ongoing Mars exploration missions closely related to astrobiology. Section 3 discusses targets for the search and analysis of astrobiology. Section 4 elaborates on the extraterrestrial life exploration method using a microscope, as well as other methods. Section 5 introduces the life signature detection fluorescence microscope (LDM) we developed for extraterrestrial life exploration.

2 Astrobiological Instruments Used/Designed for Mars and Their Findings

In this section, we review past and ongoing Mars exploration missions closely related to astrobiology. Their onboard instruments and the results of experiments and observations are summarized.

2.1 Viking

2.1.1 Mission

Viking is a Mars exploration mission conducted by NASA in the 1970s. Two spacecrafts, Viking 1 and Viking 2, successfully landed on Mars. Each Viking mission consisted of an orbiter and a lander. The lander was separated from the orbiter in the orbit and landed on Mars’ surface. While the exploration was initially planned to continue for 90 days after landing in the Viking mission, both landers and orbiters operated far beyond the designed lifetime and continued Mars exploration.

2.1.2 Instruments and Experiments for Astrobiology

A pioneering life exploration was performed by the Viking Landers. Organic compound analysis and metabolic activity measurements were performed using Mars’ surface samples, as summarized below.

(1) The thermal volatilization gas chromatography/mass spectrometry (TV-GC/MS) experiment Organic compound analysis experiments were performed with TV-GC/MS. The presence of an organic compound was expected if organisms were to exist. The samples were heated to 200 °C, 350 °C, and 500 °C, and the released gas molecules were analyzed. However, the organic compound derived from the Mars’ surface samples was lower than the detection limit (Biemann et al. 1977).
(2) The gas exchange (GEX) experiment In the GEX experiment, a nutrient solution containing amino acids and vitamins and a mixed gas containing carbon dioxide were added to the sample chamber. The gas composition was monitored through gas chromatography (GC). If organisms were to be present, the gas composition may have changed due to the release of carbon dioxide and other metabolic gases. Although carbon dioxide generation was observed, it was attributed to a reaction by an oxidizing agent (e.g., Fe₂O₃) present in Mars soil (Oyama and Berdahl 1977).

(3) The labeled release (LR) experiment A nutrient solution containing organic acids and amino acids (formic acid, glycolic acid, glycine, D-alanine, L-alanine, D-lactic acid, and L-lactic acid) labeled with radiocarbon (¹⁴C) was added to test if ¹⁴CO₂ is released by respiration. The ¹⁴C release was detected in the presence of surface samples, but no longer detected when the sample was heated at 160 °C before the reaction. This result suggested the presence of organisms (Levin and Straat 1977). However, the organic compound was below the detection limit of TV-GC/MS, and the presence of an oxidant in the soil could explain the experiment result. Hence, it was not concluded to be biological although the reaction that totally explains the label release results is not known. (Margulis et al. 1979).

(4) The pyrolytic release (PR) experiment In the PR experiment, carbon dioxide and carbon monoxide labeled with ¹⁴C were added to the sample together with water, and then irradiated with light. This experiment aimed to test if the released ¹⁴C can be detected as a result of the thermal decomposition of the organic compound produced by photosynthesis. Although a small amount of ¹⁴C was released, the same release level was observed in the experiment using the sample heated at 90 °C for 2 h. Therefore, the release was suggested to be due to a nonbiological reaction (Horowitz et al. 1977).

The organic compounds were below the detection limit of the TV-GC/MS instrument (Klein 1999). However, the sensitivity of the TV-GC/MS equipment used in Viking was re-examined after its mission. The detection sensitivity of the TV-GC/MS was not high enough, and microorganisms could not be detected in samples with low microbial density, such as those from the Atacama Desert in Chile (Navarro-Gonzalez et al. 2006). In addition, detecting nonvolatile compounds was difficult (Benner et al. 2000). Therefore, the negative conclusions of the Viking mission and the performance of another life signature search program with a higher sensitivity are worth reconsidering.

2.2 Mars Science Laboratory (MSL)

2.2.1 Mission

MSL was a Mars exploration mission by NASA, with Curiosity as its rover. The MSL was launched in 2011, and Curiosity landed on the Gale crater in 2012. The mass of Curiosity is approximately 1 t and much larger than those of the previous Mars exploration rovers, Spirit and Opportunity, which landed on Mars in 2004. Therefore, Curiosity was lowered to Mars’ surface by rocket-powered deceleration to prepare for the rover to touch down on the surface.

2.2.2 Instruments and Experiments for Astrobiology

The Sample Analysis at Mars (SAM) was a TV-GC/MS installed on Curiosity and used for organic compound exploration. The SAM analysis results closely related to astrobiology are listed below.
(1) **Methane measurement in the atmosphere** The presence of methane near 10 ppb in the atmosphere and temporal fluctuations for approximately 100 days were detected (Webster et al. 2015). This discovery is important from an astrobiology perspective because methane can be an energy source for methane-oxidizing bacteria. Methane might have been produced by the past and/or present activity of methanogen instead of or in addition to the geochemical reaction ((Atreya et al. 2007) see Sect. 3.2).

(2) **Soil component analysis** Water, hydrogen sulfide, sulfur dioxide, etc. were detected in the Gale crater soil (McAdam et al. 2014), indicating a nonequilibrium between the oxidized and reduced sulfur in the surface sample.

(3) **Measurement of organic compound in mudstone** Organochlorine compounds, such as chlorobenzene (Freissinet et al. 2015), and organic compounds, such as thiophenes and aromatic compounds (Eigenbrode et al. 2018), were discovered in approximately 3.5 billion year-old mud rocks in the Gale crater. They were obtained by excavating the rock surface. Samples were taken from mud rocks and surface sand at four locations. Comparative measurements using the SAM provided data that much exceeded the instrument-background in only one sampling site, that is, Cumberland (Freissinet et al. 2015).

The organochlorine compounds found by the SAM were thought to be formed by the reaction between the organic compounds present on Mars and chlorides, such as perchlorate, on the surface by heating during analysis (Keppler et al. 2014). In the method involving thermal decomposition, the organic compound structure before the analysis cannot be estimated. Organic compounds before decomposition may be either derived from the past living organisms on Mars or the nonliving organic compounds contained in the meteorites and interplanetary dust particles (IDPs) that have flown to Mars. Nevertheless, note that the location-dependent difference in the amount of organic compound was detected by the analyses with SAM, and that the results suggested the presence of organic compound on Mars.

### 2.3 Mars Express

#### 2.3.1 Mission

*Mars Express* is the first Mars mission by the European Space Agency (ESA). It was launched in 2003. *Mars Express* planned to observe the atmosphere and the underground structure from the orbit of Mars and lower the “Beagle 2” lander for investigation, though the landing failed.

#### 2.3.2 Instruments and Experiments for Astrobiology

Mars had large amounts of gaseous and solid water in the past. Surface rocks provide evidence that Mars used to have liquid water in the distant past. Whether or not liquid water remains on Mars has long been debated.

The radar sounder from the *Mars Express* orbiter searched for liquid water beneath the southern ice cap on Mars (Orosei et al. 2018). A liquid lake of ~ 20 km wide was discovered under hard ice in the Planum Australe area. The pressure of ice above and the dissolved salt are expected to prevent the water from freezing. The presence of underground liquid water in Mars must be considered because it will also affect future human explorations.
2.4 ExoMars

2.4.1 Mission

ExoMars is a Martian exploration mission by the ESA and Roscosmos of Russia. This project consisted of two separately launched spacecrafts. The Trace Gas Orbiter was launched in 2016, entering the Mars orbit in 2017, with its lander failing to land. The subsequent spacecraft had not yet been launched. The scientific goals of the mission are to land a rover at a site possessing past life signatures, collect samples with a drill down to 2 m depth, and search for biosignatures by analyzing them (Vago et al. 2017).

2.4.2 Instruments and Experiments for Astrobiology

The ExoMars rover Rosalind Franklin has several instruments including the Raman Laser Spectrometer (RLS) that can identify organic compounds and minerals, a TV-GC/MS that is a MS having the derivatization process of large organic molecules, and the Mars Organic Molecule Analyzer (MOMA) that is a laser desorption MS, the last is considered less susceptible to the effects of perchlorate (Vago et al. 2017). Optical isomers of amino and organic acids can be analyzed by GC/MS after derivatization. Therefore, TV-GC/MS can reveal the possible presence of life if a bias exists in the D and L forms of amino acids, similar to Earth organisms.

2.5 Mars 2020 and MSR

2.5.1 Mission

Mars 2020 is a NASA mission following MSL. The Mars 2020 spacecraft launched in July 2020 landed on Mars in February 2021. Mars 2020 landed the rover, Perseverance, on the Jezero crater on Mars. The mission aims not only to perform exploration by itself, but to take samples to Mars for future missions that would bring them back to Earth (Muirhead et al. 2020). Perseverance will complete taking samples on Mars, pack them in cylindrical containers, and store them on Mars. In the MSR plan, the containers will be picked up by a rover developed by the ESA and sent by a NASA rocket to a spacecraft waiting in the orbit around Mars for return to Earth. The returned spacecraft will come back to Earth’s orbit and drop a capsule containing a sample onto Earth in the early 2030s. The planetary protection policy on handling the returned sample from Mars has been reevaluated to cope with the possible presence of microorganisms contained in the returned samples (Craven et al. 2021).

2.5.2 Instruments and Experiments for Astrobiology

Mars 2020 is equipped with the Scanning Habitable Environments with Raman & Luminescence for Organics & Chemicals (SHERLOC) instrument which was designed based on Raman spectroscopy and ultraviolet (UV) fluorescence spectroscopy to examine the types of organic compounds and minerals (Bhartia et al. 2021). SHERLOC can analyze without heating nor destruction and is equipped with a camera. The organic compound distribution in samples can be imaged. Furthermore, information on organic compound structures, such as aromatic compounds and fatty acids, can be obtained.
In situ analysis in space exploration missions is severely restricted by limited resources, such as mass, volume, and electric power. However, if the samples can be brought back to Earth, they can be analyzed with various large analyzers in ground laboratories. MSR may lead to breakthroughs for astrobiology through an extensive analysis of the returned Mars samples.

2.6 MMX

2.6.1 Mission

The MMX mission led by JAXA is under development. It primarily aims to return samples from the Martian moon, Phobos (Kawakatsu et al. 2019; Usui et al. 2020). According to the five-year plan of the MMX flight, the spacecraft will be launched in September 2024, reach around Mars in August 2025, and return to Earth in September 2029. The spacecraft will move to a quasi-satellite orbit (QSO) around Phobos after entering the Martian orbit. The remote sensing observations of Phobos will be performed from the QSO by the onboard instruments. The spacecraft will then land and collect samples from Phobos. The targeted amount of sampling is 10 g or more. The capsule enclosing the samples will reenter Earth’s atmosphere and will land. The samples will be delivered to the ground laboratory for initial inspection and curation.

2.6.2 Relationship with Astrobiology

During the MMX planetary protection evaluation, less than $10^{-6}$ surviving microorganisms in the samples were brought back to Earth, fulfilling the planetary protection requirement. In contrast, dead Martian microorganisms may be expected (Fujita et al. 2019; Kurosawa et al. 2019). We believe that the MMX returned samples provide a valuable opportunity to access and explore Mars-related samples in ground laboratories.

3 Targets in Astrobiological Exploration on Mars

3.1 Organic Compounds

A wide variety of organic compounds can be found on Mars, including the following: (1) organic compounds abiotically formed on Mars; (2) organic compounds delivered to Mars’ surface from space; (3) organic compounds biotically formed by the past life on Mars; and (4) organic compounds biotically formed by extant life on Mars.

3.1.1 Possible Organic Compounds Formed on Primitive Mars

Substantial geological and mineralogical evidence suggest that Mars had surface liquid water (ocean) of up to 30% of the surface in its Noachian era (Forget and Hauber 2015). The presence of an ocean substantiates that Mars had a dense atmosphere during that era. If the formation mechanism of the primitive atmosphere of Mars could be similar to that of Earth, the atmosphere could be weakly reducing, where carbon dioxide and nitrogen are the major constituents with reducing species (e.g., carbon monoxide) (Catling and Kasting 2017). Forming organic compounds, especially nitrogen-containing compounds like amino acids, from a weakly reducing atmosphere is more difficult than from a strongly reducing
atmosphere (i.e., atmosphere containing methane and ammonia as major components). A nitrogen molecule, N₂, can hardly be dissociated by solar UV (Fayolle et al. 2013), which is the energy source with the highest flux on the ancient Martian surface. In spark discharge experiments simulating thundering, CO₂–N₂ and CO–N₂ mixtures were not good starting materials for forming amino acids (Kuwahara et al. 2012; Schlesinger and Miller 1983). Accordingly, thundering cannot be the mechanism producing organic compounds in ancient Mars.

Even in the weakly reducing atmosphere of Mars, it was possible to synthesize organic compounds, including amino acids or their precursors. Amino acid precursors were formed when the weakly-reducing gas mixtures of CO₂, CO, N₂, and H₂O were irradiated with high-energy particles, which simulated cosmic rays (Kobayashi et al. 1998). Amino acid precursors are compounds that yield amino acids after hydrolysis. Meteor-impacts could be another possible energy source for the amino acid formation. Miyakawa et al. (1999) and Miyakawa et al. (1998) conducted high-temperature plasma experiments that simulated plumes after bolide impacts in the CO–N₂–H₂O-type atmosphere and found amino acid precursors and nucleic acid bases in the products. A trace amount of glycine could be formed by shock synthesis in the CO₂–N₂–H₂O–Fe system (Takeuchi et al. 2020). Accordingly, amino acid precursors are expected to be formed in Noachian Mars and Hadean Earth, both had weakly reducing atmosphere.

The amino acid precursors formed in the CO₂–CO–N₂–H₂O-type gas mixtures were not simple molecules, such as aminonitriles. They were complex molecules with large molecular weights larger than hundreds (Kobayashi et al. 1997). Organic compounds would have been altered during long-term preservation in Mars environments. Most organic compounds would have been altered to more stable complex molecules, such as hydrocarbons and kerogen-like materials. However, free amino acids were less stable against UV light and radiation than bound amino acids (Kobayashi et al. 2004; Takano et al. 2004a); hence, we can expect that amino acids might have survived in complex precursor forms rather than free amino acids in such environments as the Mars subsurface protected from radiation.

3.1.2 Organic Compounds Delivered from Space

Various organic compounds have been detected in extraterrestrial bodies, e.g., carbonaceous chondrites (CCs) (Kvenvolden et al. 1970) and comets (Kissel and Krueger 1987). CCs contain a wide variety of organic compounds, including bioorganic compounds like amino acids (Kvenvolden et al. 1970), nucleic acid bases (Martins et al. 2008), and sugars (Furukawa et al. 2019), suggesting that exogenous organic compounds could be important sources for the life on Earth or on other planets in the early evolutionary stages. The amino acid concentration in CCs increases after acid hydrolysis (Glavin et al. 2006), showing that both free amino acids and amino acid precursors are present in CCs.

The organic compounds found in CCs could have been formed in molecular clouds (Greenberg and Li 1997) and meteorite parent bodies (Kebukawa et al. 2013). Amino acids were formed in the experiments, where interstellar ice analogs were irradiated with high-energy protons (Kasamatsu et al. 1997) or UV light (Bernstein et al. 2002; Caro et al. 2002). Amino acids were formed when an aqueous solution of formaldehyde, glycolaldehyde, and ammonia was heated, simulating the interior of meteorite parent bodies (Kebukawa et al. 2017). Experiments simulating extraterrestrial environments suggested that amino acid precursors, rather than free amino acids, were formed in space. Aminonitriles (Glavin et al. 2020) and hydantoins (Shimoyama and Ogasawara 2002) were proposed as candidates for extraterrestrial amino acid precursors. In addition to these simple amino acid precursors,
complex amino acid precursors were found in the product after the proton irradiation of interstellar media analogs (Takano 2004).

Exogenous organic compounds could have been delivered to primitive Earth and Mars by meteorites and comets. IDPs are other candidate carriers of extraterrestrial organic compounds. IDPs are tiny dusts suggested to originate from asteroids or comets (Brownlee 2001). Chyba and Sagan estimated the feeding rates of organic carbons to Earth by meteorites, comets and IDPs at the time about 3.8 billion years ago (Gya) and are approximately $10^2$ kg/yr, $10^4$ kg/yr and $10^7$ kg/yr, respectively (Chyba and Sagan 1992). Note that a major part of the organic carbons found in CCs are not soluble organic compounds, but are insoluble organic compounds (IOMs) (Alexander et al. 2017). We may be able to find meteoritic amino acid precursors together with IOM on Mars.

Although IDPs could have delivered more organic carbons than CCs, how much amino acid precursor IDPs could have been delivered to Earth and Mars remains unclear because most of the IDPs (or referred to as micrometeorites if they were found in terrestrial ices) were collected in the terrestrial biosphere, where IDPs could be easily contaminated by amino acids from the environment. In the Tanpopo Mission, which is the first astrobiology space experiment in Japan, the capture of IDPs was attempted in the Exposed Facility of the Japanese Experimental Module (JEM-EF) of the International Space Station (ISS) from 2015 to 2019 (Yamagishi et al. 2014). The captured dusts are now being analyzed.

We can discriminate the meteorites and Mars rocks that landed on Earth based on rare-gas content analysis. However, IDPs and meteorite powders formed by weathering would have been well mixed with Mars soil. Thus, discriminating exogenous organics supplied from the meteorites and IDPs from Mars pristine organics would be difficult when analyzing Mars soil.

### 3.1.3 Organic Compounds of Biological Origins

If carbon-based extant living organisms thrive on Mars, they would biotically produce organic compounds. Living organisms are expected to be composed of cells that contain catalytic, self-replicating, and boundary molecules, as discussed in Sect. 3.2. Terrestrial organisms use proteins as catalytic molecules, nucleic acids, ribonucleic acid (RNA), and deoxyribo nucleic acid (DNA) as replicative molecules, and phospholipids as boundary molecules. We have no information on the possible organisms on Mars. If terrestrial and Martian organisms have the same ancestors, which means that the interplanetary transfer of organisms between Earth and Mars happened, a Martian organism would use the same kinds of bioorganic compounds like DNA. If not, we should think that Martian organisms would use different kinds of biomolecules from the terrestrial counterparts.

Whether or not it is true that terrestrial and Martian organisms have the same ancestor, we think that Martian organisms might use proteins or polymers of amino acids because (1) proteins are quite multipotent molecules, including catalytic ability; and (2) amino acids are very easily formed in abiotic environments, including space environments (see Sect. 2.1.2).

We can judge whether the amino acids in environments are biogenic or abiotic (Fig. 1). Terrestrial organisms use only 20 kinds of amino acids (common amino acids). If the amino acid types used in the terrestrial amino acid are detected, it may represent terrestrial life. Another signature of biotic amino acids is that large complex protein amino acids (e.g., histidine, tryptophan, arginine, and lysine) were rarely found in CCs or abiotic synthesis products (Glavin et al. 2020). More than 70 kinds of amino acids were found in CCs. Most of these were not found in terrestrial organisms. The presence of amino acids specific to CCs suggests the meteorite origin of organic compounds.
Fig. 1 Flowchart for judging the origin of the organisms on Mars through amino acid analyses. Amino acids often present as polymers can be extracted by acid hydrolysis and analyzed (Enya et al. 2021). If the amino acids could not be detected from the organism in question, the organism is judged to have an extraterrestrial life or nonlife form. Extracted amino acids are analyzed to test if they are standard terrestrial common amino acids or not. If the amino acids are different from the terrestrial proteinogenic type, they will be tested to determine if they are those often contained in a meteorites. If the answer is “yes,” the organism is a nonlife originating from a meteorite. If the answer is “no,” the organism is likely to depict a nonterrestrial life. The organism is likely to be a life form if the organism contains a terrestrial-type amino acid. DNA and other analyses will be performed to judge if the organism is a terrestrial or nonterrestrial life.

Terrestrial organisms use L-enantiomers, except for achiral glycine. In contrast, abiotically formed amino acids are fundamentally racemic mixtures, although small enantiomeric excesses could sometimes be found in CCs (Cronin and Pizzarello 1997). The reason why meteoric amino acids have such enantiomeric excesses is unclear, but many hypotheses have been tested on how such enantiomeric excesses were formed (Takahashi and Kobayashi 2019). The L- and D-forms are catalytically equivalent. The D-form can constitute a living organism. Accordingly, the presence of organic compounds consisting only of D-form amino acids is strong evidence of nonterrestrial life (Fig. 2).

Terrestrial organisms use DNA and RNA as their replicative genetic material, that is, RNA in specific viral particles and DNA in the remaining living organisms. Terrestrial DNA and RNA contain five bases (i.e., adenine, cytosine, guanine, thymine, and uracil), two sugars (ribose and 2-deoxyribose), and phosphates as building blocks. DNA and RNA can be extracted and partially purified by the procedure, including physical and/or chemical cell disruption and protein and lipid elimination, followed by simple liquid chromatography. These are done by skilled scientists with sufficient knowledge and experience of the techniques. However, the presence of DNA and/or RNA can be easily identified by fluorescence microscopy using the DNA- and/or RNA-specific fluorescence pigments.

We are not sure if Martian organisms might use such nucleic acid-type replicative molecules or not. If nonterrestrial genetic materials are to be found, these would suggest the presence of a nonterrestrial organism. If Martian organisms have a different ancestor as the terrestrial ones, they might use different genetic systems, unless there are compelling unknown biophysical reasons to use the same system. Even if Martian organisms use DNA–RNA-like systems with Watson–Crick-type base pairing, their base and sugar may be different from ours. Cafferty et al. (2018) discussed 91 RNA base analogs, including four canonical bases (i.e., adenine, cytosine, guanine, and uracil), and concluded that several alternative bases can replace the canonical bases. Regarding sugars, ribose has so many isomers (e.g., arabinose) and analogs (e.g., ketopentoses, tetrose, hexoses, and amino sugars).
Fig. 2 Flowchart of judging the origin of Mars organisms through an analysis of the enantiomeric excess of amino acids. Amino acids often present as polymers can be extracted by acid hydrolysis and analyzed (Enya et al. 2021). If amino acids could not be detected from the candidate organism, the organism is judged as an extraterrestrial life or nonlife form. The extracted amino acids are analyzed to test the enantiomeric excess. The organism is nonlife if the amino acids are not L- or D-form (i.e., racemic mixture) and likely to be a nonterrestrial life if they are D-form. In L-form, the organism is likely to be life, and DNA and other analyses will be done to judge whether the organism is terrestrial or nonterrestrial life that can construct nucleosides; hence, we will not be surprised if Martian organisms use noncanonical sugar.

If the Martian organisms would use the same DNA-based genetic system, a very sensitive detection technique can be applied for the analysis, which is called the polymerase chain reaction (PCR) method. If Martian organisms and terrestrials would have a common ancestor, the PCR could be the most promising technique of detecting them. However, the PCR needs the sequence information to design the primers it uses. Accordingly, the PCR is applicable only to organisms with a sequence similar to that of the terrestrial ones. If the DNA could be amplified by PCR and the phylogenetic tree could be constructed from the gene sequence, based on the phylogenetic tree, we might be able to tell whether the life on Earth may be transferred from Mars or opposite, and when the Martian organism was transferred from Earth if the latter is the case (i.e., billions of years ago or very recently). The genetic material sequence being unrelated to terrestrial organisms suggests the presence of an extraterrestrial organism (Fig. 3).

If Martian organisms would have been extinct, their organic compounds would have altered in the Mars regolith, rocks, or underground. Amino acids would have been decomposed or racemized. Accordingly, both the concentration and the enantiomeric excesses of the amino acids from extinct organisms would decrease (Takano et al. 2004b), and it becomes difficult to differentiate them from the abiotic ones, a long time after extinction. The most prominent biomolecules may be hydrocarbons mainly altered from membrane lipids because hydrocarbons are quite stable. Biphytanes and hopanes are assumed to be the chemical fossils of archaea and bacteria, respectively. These remain billions of years after their death on Earth (Hunter 2013). Hydrocarbons with special structures are promising targets to be searched for extinct life.

The last evidence for extinct (and extant) life is the carbon isotope ratio. Carbon has two stable isotopes, namely 12C and 13C. Their ratio is expressed as follows by the $\delta^{13}C$ value:

$$\delta^{13}C = \left[ \frac{^{13}C/^{12}C}_{\text{sample}} / \left( \frac{^{13}C/^{12}C}_{\text{standard}} - 1 \right) \right] \times 1000\permil$$
Fig. 3 Flowchart of judging Mars organisms from DNA analyses. DNA is extracted from the sample (Enya et al. 2021). If DNA could not be extracted, the organism is not life or nonterrestrial life; otherwise, the negative result may be attributed to the technical problem. RNA analysis or other analyses will tell which is the case. If DNA is extracted, the nucleic acid bases will be analyzed. If the bases are adenine, cytosine, guanine, and thymine, the PCR analysis will be performed to test if the DNA strand can be amplified or not. If the DNA strand is amplified, the sequence is analyzed. If the DNA sequence is read, the phylogenetic tree is constructed. If the tree is constructed, the position of the sequence obtained in the tree will tell how the organism is related to the terrestrial organisms. The Mars organism may originate from the terrestrial organism transferred from Earth some time ago. The approximate time of transfer can then be inferred. Contrarily, the Mars organism may be transferred to Earth before the diversion of the Domains Archaea and Bacteria, although the transfer direction may be opposite. In all analyses from the base to the phylogenetic tree construction, the negative result suggests that the organism found on Mars is nonterrestrial life, although we cannot reject the possibility that the failure is caused by the technical problem.

The carbons of bioorganic compounds have lower (minus) \( \delta^{13}C \) values than those of inorganic carbons because biological processes incorporate lighter isotopes more into biological systems. This phenomenon has been applied to search for evidence of the oldest life (Ivlev 2001). These techniques can be used to identify possible life by analyzing the carbon isotope ratio and comparing it with those of inorganic carbon.

3.2 Microorganisms: Catalysis, Molecules, and Structures

In addition to the organic component, the cellular structures and several characteristics of microorganisms, especially living microorganisms, can be targets for exploration on Mars’ surface. The form, constituent, and characteristics of life must be considered when we search for living cells or signatures of life. The most significant characteristic of terrestrial life is that it is built of “cells” that are “organic components surrounded by membranes.” Cells of terrestrial life are surrounded by a semipermeable membrane, called the cell membrane. The necessity of the cell membrane is closely related to “metabolic reactions.” All cellular activities are maintained by the complex network of continuous chemical reactions, called the metabolic reaction. The reaction is maintained and enhanced by keeping the catalysts, reactants, and products at high concentrations. This is achieved by the membrane impermeable to these components.
Table 1 Molecular composition of an *Escherichia coli* cell (Watson 1976)

| Molecule      | Composition (%) |
|---------------|-----------------|
| Water         | 70              |
| Protein       | 15              |
| Nucleic acid  |                 |
| DNA           | 1               |
| RNA           | 6               |
| Lipid         | 3               |
| Carbohydrate  | 4               |
| Mineral       | 1               |

The second characteristic of terrestrial life is the metabolic reaction itself. Most of the metabolic reactions of terrestrial life are catalyzed by enzymes; thus, catalytic reactions can be used as the life signature targets.

The third characteristic comprises organic compounds constituting cells. Table 1 shows the molecular composition of *Escherichia coli*, the model organism used in biology.

The molecular content of *E. coli* is similar to those in all living organisms, including higher animals and plants. The cell consists of approximately 70% water and 30% organic compounds. Terrestrial life is made of various organic compounds, such as nucleic acids, proteins, and lipids. Organic compounds can be the target for the search for life.

When we consider possible Martian life, the similarities between early Earth and Mars are noticed. On early Earth, oxygen was absent for about four billion years, and the carbon dioxide concentrations were at least 100–1000 times higher than the present level (Kasting 1987). In addition, liquid water was present on the surface (Ernst 2007). Early Mars would also have a dense carbon dioxide atmosphere, a wet and warm climate (Pollack et al. 1987), and oceans on the surface (Carr and Head 2003). This similarity led us to estimate a similar type of life on Mars as that on Earth.

Amino acids could be used for Martian life because they have been found in meteorites and can be considered ubiquitous in the universe, as discussed in Sect. 3.1. Therefore, protein-like organic compounds, which are polymerized amino acids, are our essential targets. It took approximately 2 billion years for eukaryotes with a large cellular structure to appear on Earth, evolving from prokaryotes with simpler cellular structures. The warm and humid periods on Mars were much shorter than those on Earth. Accordingly, life may remain in the prokaryotic stage on Mars. Prokaryotes on Earth do not have intracellular transport systems; therefore, substances within a cell were transferred only by diffusion. Accordingly, the cell diameter in most prokaryotes does not exceed 1 µm. Based on the cell size estimation on Mars, which is still in the prokaryotic stage, high-resolution microscopes are effective tools for detecting single-cell prokaryotes.

The possible presence of a viable microbial community in the Martian subsurface has been postulated because higher temperatures and pressures can stably sustain liquid water at depths below a few kilometers (Chapelle et al. 2002; Clifford et al. 2010; Michalski et al. 2013). Microbial energy sources, such as molecular hydrogen, may also be present there (Atreya et al. 2007). Molecular hydrogen has been used as an energy source for a wide variety of chemolithoautotrophic microorganisms that obtain chemical energy from reduced compound oxidation. Molecular hydrogen could be produced in the Martian subsurface by serpentinization (Atreya et al. 2007). Serpentinization is a reaction of olivine-and pyroxene-rich rocks with liquid water (Schulte et al. 2006). Methanogens, which are methane-producing microorganisms, use molecular hydrogen as their energy source. Methane has been reported in the Martian atmosphere (Webster et al. 2018). Although the origins of methane are uncertain, several methane generation processes have been proposed,
including biotic and abiotic processes (Atreya et al. 2007; Etiöpe et al. 2011; Oehler and Etiöpe 2017). Earth has approximately 80% of natural emissions of methane originating from methanogens (Etiöpe et al. 2011); thus, Martian methane may also be produced by methanogens.

Accumulating information on the environment of the current Mars suggests the possible presence of microorganisms near the surface, although current Martian environments are hostile to life. Terrestrial microorganisms have expanded their habitats by adapting to the environment. They have spread their habitat everywhere, including polar regions, hot springs, and deserts. The limits of survivable and/or proliferating conditions are within the Martian environmental factors on Mars’ surface (Yamagishi et al. 2010). For example, microorganisms that metabolize at $-20 \, ^\circ\mathrm{C}$ have been found in the Siberian permafrost (Rivkina et al. 2000). Some microorganisms can also grow under the following simulated Mars conditions: low temperature ($0 \, ^\circ\mathrm{C}$); low pressure (7 hPa); and anoxic CO$_2$-dominated atmosphere (Nicholson et al. 2013). A radiation-tolerant microorganism, called *Deinococcus radiodurans*, can survive 5 kGy without loss of viability (Cox and Battista 2005; Dartnell et al. 2007). The dose is much higher than the total dose of ionizing radiation on the Martian surface (76 mGy y$^{-1}$) measured by Curiosity (Hassler et al. 2013). The Martian surface sample contains 0.4–0.6% perchlorate (Hecht et al. 2009), a strong oxidant when heated. Some microorganisms are resistant to perchlorate (Shcherbakova et al. 2015) and utilize it as an electron acceptor for energy production (Oren et al. 2014). UV radiation is harmful, and terrestrial microorganisms cannot survive when directly exposed to the Martian surface. However, UV would be shielded by thin layers (less than a millimeter) of dust (Mancinelli and Klovstad 2000); thus, microorganisms could survive at a depth of several centimeters from the surface.

Possible energy sources have also been found near the surface, including methane (Webster et al. 2018), reduced iron, and sulfur (Grotzinger et al. 2014). Some methane-oxidizing bacteria on Earth use manganese dioxide, iron hydroxide, and sulfate as electron acceptors (Beal et al. 2009), which are known to exist on Mars. The seasonal efflux of liquid water is postulated in the middle and high latitudes of Mars, which is called the recurring slope lineae (RSL), where narrow dark streaks appear during warm seasons on the steep slopes of craters (McEwen et al. 2014). Although the reason for the formation process of the RSL remains unclear, it could be the flows of the dry granular dust (Dundas et al. 2017) or the liquid water flow (McEwen et al. 2014). Hydrated salts of magnesium perchlorate, magnesium chloride, and sodium perchlorate were observed at some flow sites (Ojha et al. 2015), which lowered the freezing point and kept water in a liquid state in Martian environments. The radar sounder on Mars also recently detected underground salt water beneath the southern ice cap (Orosei et al. 2018). Some microorganisms on Earth are known to grow in highly concentrated salt solutions (Grant 2004); hence, these halophilic microorganisms could be found in Martian briny environments. These findings suggest that microorganisms may be found near Mars’ surface and can be attractive targets for life explorations.

### 4 Microscopy and Other Methods

#### 4.1 Microscopy

#### 4.1.1 Optical Microscope

Microscopes, especially optical microscopes, are one of the most important instruments in biology. Robert Hooke observed the cork, discovered chamber-like structures, and named them “cells” (Hooke 1665).
Fig. 4 Schematic view of microorganism detection using a fluorescence microscope. The sample is transferred by the robotic arm of the rover and introduced to the sample holder. After adding the fluorescence pigment solution, the sample images are taken through the bottom window with a fluorescence microscope, illuminating the sample with light from the excitation light source. The images are then captured by a sensor. A high-contrast image will tell the morphology and characteristics of the organisms depending on the staining specificity of the fluorescence pigment. In this fluorescence image, structures containing organic compounds surrounded by the membrane were stained green, while those surrounded by permeable membranes were stained red in the presence of the fluorescence pigment mixture of SYTO 24 and propidium iodide (PI). The green and red cells represent the living and dead cells, respectively, of *Escherichia coli*. This figure is produced by modifying a figure in Enya et al. (2021)

However, if a normal optical microscope is used as an image magnifier, for example, for life exploration on Mars, it will be very difficult to distinguish microorganisms from a micrometer-sized particle with irregular shape, size, and composition. Nonfluorescent pigments, such as Trypan blue, Safranin, and Crystal violet, are often used to stain biological components. With these pigments, the biological components can be stained in a specific color and differentiated from nonbiological particles. However, a higher contrast between the background and the microorganisms is usually required to detect and identify microorganisms in samples for life exploration.

Fluorescence microscopy provides a higher contrast than simple staining. Figure 4 shows the principle of microorganism detection in soil using a fluorescence microscope and the flow of the procedure on Mars. First, the surface sample is acquired by a sampler mounted on a rover. Next, the fluorescence pigment solution is added. This fluorescence pigment binds to the molecules with specific characteristics in microorganisms. By irradiating it with excitation light, pigments selectively bound to microorganisms emit fluorescence. The high-contrast fluorescence image in a dark field can be detected with a fluorescence microscope.
Numerous fluorescent pigment variations exist (Table 2). Each fluorescent pigment has the specificity to bind to components with specific characteristics. Therefore, fluorescent pigments not only enhance the contrast and enable the identification of microorganisms, they also enable the identification of specific microorganism characteristics. As will be described in the subsequent sections, the fluorescent pigments in our current basic plan are SYPRO Red for the organic compound detection, a mixed pigment of SYTO24 and PI for the cell membrane detection, and CFDA-AM to detect catalytic reactions (Enya et al. 2021; Yamagishi et al. 2010; Yoshimura 2019).

An image obtained with a fluorescence microscope provides information about the shape, size, and uniformity of objects. This information is useful when judging whether or not it indicates life, because microorganisms in the same species are well known to be similar in shape and size usually. Although the microbe cell size is often less than 1 µm, it can easily be detected by a high-contrast image of fluorescence in a dark background.

In life exploration processes using a fluorescence microscope, false positives must be reduced. The images of the sample taken before the addition of the pigment solution can facilitate to identify the false positive signal, the fluorescence from mineral particles, for example. Section 5 provides more details on our fluorescence microscope system.

### 4.1.2 Electron Microscope

An electron microscope utilizes an electron beam instead of light used in an optical microscope. Two types of electron microscopes are often used: transmission electron microscope (TEM) and scanning electron microscope (SEM). An important advantage of electron microscopy over light microscopy is a much higher spatial resolution. However, adopting an electron microscope for the sampled soil for *in situ* life exploration is difficult because of the small observable area. An electron microscope has the potential to be used for returned samples after selecting the specific target of interest in a ground laboratory.

### 4.1.3 Scanning Probe Microscope

A scanning probe microscope is an instrument that utilizes a probe with a sharp tip to trace the surface of an observation target. We can obtain magnified three-dimensional images by scanning the sample’s surface. Various types of scanning probe microscopes are available for use, including the scanning tunneling microscope that uses a tunnel current and the atomic force microscope that uses interatomic force. The latter was onboard Phoenix lander (Kounaves et al. 2009) and used to observe a mineral particle.

When considering applications for life exploration, the scanning probe microscope has advantages and disadvantages that are similar to those of an electron microscope. A scanning probe microscope provides a very high spatial resolution compared with an optical
microscope, but its observable area is small. The scanning probe microscope can be used for returned samples in a ground laboratory.

4.2 MS

4.2.1 Use of Mass Spectrometers in Space

MS is one of the most promising methods for analyzing organic compounds in extraterrestrial environments. Most spectrometric techniques provide information on the structure of the targeted molecules, such as the presence of specific functional groups. Thus, identifying complex organic molecules using most spectrometric methods is not easy, unless a comparison of the spectra of authentic standards and the sample is possible. In contrast, MS can provide information on the molecular weight and structure of the target molecule, by which we may be able to identify or infer the molecule without authentic standards.

Mass spectrometers are composed of sample inlets, ion sources, analyzers, and ion detectors. MS can be applied to molecules that can be ionized. Hard and soft ionization are the two types of ionizing methods that can be employed. Typical hard ionization is electric ionization, by which the fragments of the target molecule are formed and analyzed. Accordingly, the molecule structure can be estimated by hard ionization. Various soft ionization techniques have recently been developed, minimizing the target molecule fragmentation and helping estimate its molecular weight. Soft ionization methods include matrix-associated laser desorption/ionization (MALDI) and electrospray ionization (ESI), and both of which can be applied to such large molecular weight compounds as proteins and DNA. These methods have not been used in planetary explorations due to the difficulty of handling samples in space. For example, in MALDI–MS, solubilized samples must be mixed with the matrix, applied to the sample holder, dried, and analyzed. Alternatively, the solid sample must be applied to the sample holder, covered with the matrix, dried, and analyzed.

The original analyzer for mass spectrometers is a magnetic sector-type analyzer, which is relatively heavy. Lighter-weight analyzers, such as time-of-flight (TOF) analyzers, are often used in space (Kissel et al. 2003). In the case of a TOF analyzer, a longer ion path is essential for a better ion resolution. A multiturn time-of-flight mass spectrometer (MULTUM) is a promising lightweight and high-resolution instrument, in which infinite flight paths of ions in a chamber are achieved by using a space and time-focused closed flight orbit (Shimma et al. 2010).

When Comet Halley was approaching Earth, a mass spectrometer, called PUMA, on board Vega 1 analyzed the cometary dusts in the coma (an envelope around the nucleus of a comet), where the impact ionization technique was applied. Ionized molecules and those ones formed upon a hypervelocity impact on the PUMA target were introduced and analyzed. The cometary coma appeared to contain a wide variety of complex organic compounds (Kissel and Krueger 1987). On a mission to the Saturn system, the Ion and Neutral Mass Spectrometer (INMS) on board Cassini analyzed plumes that erupted from Enceladus, a Saturn satellite, where dual ion sources (open and closed) and a quadrupole mass analyzer were equipped. The analysis showed the presence of liquid water and organic compounds in its subglacial ocean (Waite et al. 2006).

4.2.2 Hyphenated MS

If the target sample is a mixture of complex organic compounds, identifying individual compounds is difficult due to the mass spectrum complexity. To analyze the mixture of complex
organic compounds, the sample must be separated by other methods (e.g., chromatography) prior to MS. MS of this type is referred to as hyphenated MS. The typical ones are gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS). LC/MS is a popular technique for analyzing mixtures of complex molecules like bioorganic compounds in ground laboratories because most of these molecules are less volatile and soluble in water or organic solvents. However, it cannot be easily applied to planetary exploration. A liquid chromatograph system is not designed for use in extraterrestrial environments with different gravities and atmospheres from Earth. Liquid handling in vacuum or low pressure can be the problem in such cases.

Meanwhile, GC/MS has been used in space since the 1970s. MS is an analytical method for gaseous molecular species; hence, the combination of GC and MS is relatively easy. However, nonvolatile compounds must be modified to a gaseous form by derivatization or pyrolysis. In the Viking mission, GC/MS was used to analyze the gaseous compounds in the atmosphere, in the gaseous product of the surface sample after pyrolysis, and in the gaseous product after the GEX experiments in one of the three Viking biology experiments (Rushneck et al. 1978). The gas chromatograph–mass spectrometer (GC-MS) of Viking used a magnetic sector-type analyzer.

NASA has been using a GC-MS with a pyrolyzer in their subsequent Mars projects, such as MSL. Its Curiosity Rover had a chemical analysis module, called SAM, equipped with a GC and a quadrupole mass spectrometer. Nonvolatile samples in the Martian surface samples were introduced to the GC-MS after pyrolysis or derivatization. In 2018, the presence of complex organic compounds was reported after the analysis of mudstones at the Gale crater by TV-GC/MS (Eigenbrode et al. 2018).

Although TV-GC/MS is a good technique for characterizing polymers or polymer-like materials, identifying individual compounds, such as bioorganic compounds, is difficult. Chemical derivatization is essential in identifying molecules like amino and nucleic acids by GC/MS. As an amino acid has two or more hydrophilic groups. It must be converted to a hydrophobic group before being injected to GC. One of the most popular and promising derivatization methods for amino acids is esterification/acylation, where amino acids were converted as follows (Gamerith 1983):

$$\text{NH}_2\text{-CHR-COOH} \rightarrow \text{R}^+\text{CONH-CHR-COOR}^-,$$

where $\text{R}^+$ is CF$_3$, C$_2$F$_5$, etc., and $\text{R}^-$ is C$_3$H$_7$, C$_4$H$_9$, etc. This method involves two-step derivatizations and is difficult to automatically process in space. A ready-to-process, one-step derivatization method like the trimethylsilylation method (Sobolevsky et al. 2003) should be considered.

Most amino acids on geological samples are combined amino acids (or amino acid precursors) instead of free amino acids, as discussed in Sect. 2.1. Because the direct analysis of the combined amino acids is difficult, these should be hydrolyzed to liberate the free amino acids before analysis. In the laboratory, proteins and other forms of combined amino acids are hydrolyzed in 6 M HCl at 110 °C for 24 h (Mustațea et al. 2019). The conditions cannot be easily applied in the automatic analysis in extraterrestrial environments. Alternatively, hydrolysis with solid acid catalysis (Masuda and Dohmae 2010) was developed.

Another technique used for the biological molecules in complex large molecules is reactive TV-GC/MS (Ibrahimi et al. 2020). In this method, tetramethylammonium hydroxide is added to pyrolizers, and the target molecules are derivatized during pyrolysis to small molecules, which is characteristic of a biological monomer. This reaction was adopted in the GC-MS onboard the Curiosity Rover (Williams et al. 2019).
4.3 Various Spectroscopy

4.3.1 Use of Various Spectroscopy in Space

Various spectroscopy is the most fundamental technique. Chromatography requires an authentic sample, while spectroscopy does not. Most spectrometric techniques provide information on the structure of the targeted molecules, such as the presence of specific functional groups.

Spectroscopy is classified depending on the wavelength range. X-ray spectrometry is used for atomic-level analysis. The interaction of the targeted atomic species with a neighboring atom can be analyzed by X-ray spectrometry, such as X-ray absorption near-edge structure (XANES) spectroscopy and extended X-ray absorption fine structure (EXAFS). However, the X-ray source often using synchrotron radiations is not suitable for the space instrument.

UV and optical spectrometry are the most common techniques used in ground laboratories. These techniques are employed to analyze and quantify molecules, which are usually (partially) purified or used after the target-molecule-specific colorimetric reactions. Although these instruments can be made compact, the techniques cannot be easily applied to analyze the mixture of the compounds with absorption in the wavelength range of interest.

Infrared and Raman spectroscopy detect the functional groups in molecules and can be applied to mixtures and complex molecules. Among others, hollow cathode ion lasers are used for the UV micro-Raman spectrograph (Storrie-Lombardi et al. 2001). Imaging is accomplished by visible illumination and deep UV laser-induced excitation of visible wavelength fluorescence targeting unstained microorganisms. This makes it possible to take images of regions with native fluorescence, followed by the deep UV resonance Raman spectroscopy of fluorescent sites. The Perseverance Rover of Mars 2020 carries the SHERLOC with imaging ability (Bhartia et al. 2021), while the Rosalind Franklin Rover of ExoMars has RLS with an imaging ability (Vago et al. 2017).

4.4 Searching and Analyzing Activity and Other Features

4.4.1 Proliferation

Replication and proliferation are important characteristics of “living” organisms; therefore, they can be life signature targets. Combinations of sampling and culturing are often used in ground laboratories when microorganisms are considered as targets. Turbidity measurement and flow cytometry are used in laboratories to monitor and test the growth of microorganism in liquid media after inoculation. Culturing on a solid medium is another usual procedure. The number of colonies on a solid medium is counted after incubation. The shape, size, color, and changes of colonies are visually observed after incubation. The colonies appearing on a solid medium can be observed with a normal optical camera without any extra special equipment.

For life exploration, it is promising to obtain samples from underground, under rocks, or in ejecta from springs, where the irradiation of UV and/or cosmic rays is shielded. On the other hand, it is true that life adapts to the environment through evolution, and proliferated microbial cells may survive during the transferring process in the atmosphere. Therefore, the aerosol in the atmosphere or on Mars’ surface can also be samples for examination.

The Japanese Tanpopo team conducted an experiment to expose the radioresistant microorganism Deinococcus radiodurans outside of the ISS for 3 years (Kawaguchi et al.
The sample was brought back to the ground and examined after exposure, and survival of the microorganism was confirmed (Kawaguchi et al. 2020). Microorganisms are dormant as dried cells in harsh environments. Activity can be regained when the cells are placed in a better environment.

The sampling mechanism can be very simple, compact, and lightweight if the microorganisms in the aerosol are targeted. It can be done on a lander without a rover, a drill, nor a sampling arm.

One of the serious difficulties in culturing method is the selection of adequate media and conditions for culture. Microorganisms on Earth are difficult to cultivate, and the proportion of microorganisms that can be cultivated is 1% or less of the species present in the sample (Amann et al. 1995; Pham and Kim 2012). To overcome the problem, it is usually needed to test more than tens of thousands of combinations of culture media and conditions systematically. It should be mentioned that the success rate of culture less than 1% is even after these efforts. Therefore, it is challenging and critically important to determine the culture media and the conditions in planetary exploration missions, where the resources that can be carried are strictly limited.

Culturing microorganisms while maintaining the environment conditions of the obtained sample may be one of the methods for overcoming the above-mentioned hurdle. If microorganisms proliferate in the sampling place, nutrients should also be there. Accordingly, sample incubation and monitoring after water addition can be used to test the growth of the microorganisms present in the sample. In addition, the slide glass immersed in liquid water or any liquid present in the environment can be utilized for observation to find the colony of the microorganisms that attached and grew on the slide glass. The method has been used to search for the living microorganisms in hot springs (Brock 1978).

The possible contamination of the microorganisms carried from Earth is another serious issue. This contamination must be carefully evaluated in the in situ culture method. Therefore, combining careful sterilization before launching with in situ sterilization may be effective.

### 4.4.2 Detection of Death

Although there is no consensus of the definition of life, considering the universal features common to terrestrial life is useful in searching for extraterrestrial life. It is one of the universal features of terrestrial life to die or to have the ability to die. It is also considered universal, at least for now, that death in terrestrial life is a one-way phenomenon. Even if all the substances that made up life are preserved, life once dead will never be revived. Therefore, we list detection of death as a testing method.

The features of the death of unicellular microorganisms are somewhat different from those of larger multicellular organisms. Nevertheless, heat, UV rays, radiation, and other environmental condition changes can cause irrecoverable loss of their biological function. Microorganisms that are dormant for a long time can also die.

The judgment of life or not can be ambiguous because of noises and/or the limitation of instruments when candidates of the life signature are to be found by in situ exploration of microorganisms. It will help to judge the characteristics of the sample to intentionally sterilize a part of it and to perform a comparative experiment. Sterilization by heating with heater and UV radiation exposure can be done easily without any complex equipment. Other chemical or physical sterilization methods are also conceivable. Sterilization and comparison methods should be performed in combination with various other experiments already described in the previous sections.
4.4.3 Shape, Color, Growth, and Movement

In microbiology, morphology of the cells (such as shape, color and size), growth and movement are the fundamental factors used for the species identifications. Observations of the shape, color, size, growth, and movement of organism could be incorporated in the extraterrestrial life exploration.

Allan Hills 84001 (ALH84001) (McKay et al. 1996) is a fragment of a meteorite originating from Mars, which was collected in Antarctica. A fossil-like structure of microorganisms, which was found inside the meteorite, had been discussed to be possible evidence of extraterrestrial life. However, arguments against the structure as a fossilized organism have also been presented: the size of the structure is too small to contain genes, and the diameter and the length of the unit section are not uniform. Accordingly, no clear conclusion has been reported to date (Anders et al. 1996; Harvey and McSween 1996; Treiman 2003).

Numerous mushroom-shaped constructs on Mars and their growth have been reported based on the photographs taken by the Opportunity Rover at the Eagle crater on the Meridiani Planum (Joseph et al. 2019). Although the structures have not been concluded to be life, the possibility that their constituents are photosynthetic organisms or hematite has been discussed.

A microscope is effective for observing the shape, growth, and movement of microscopic organisms, and a normal camera is sufficient for macroscopic objects. The active movement of target organism unrelated to the movement of the media surrounding it suggests the endogenous moving-mechanism of the organism. For example, the continuous movement unrelated to the flow of liquid media can suggest the organism to be living. Monitoring the colonies appearing on solid media with an optical camera can provide the sufficient evidence supporting the presence of microbiological organisms in the environment. Multicellular organisms larger than microorganisms sometimes show a left–right symmetry in shape and spontaneous movement. Because it needed two billion years for macroscopic organisms to appear on Earth, we are not sure if macroscopic organisms could be evolved on Mars and other extraterrestrial solar system bodies. Nevertheless, to obtain observation-based facts, it is worth observing macroscopic shape, color, growth, and movement as our life exploration options. Both macroscopic organisms and macroscopic structure caused by microscopic organisms are targets of observation. Such observation can easily be done with a normal optical camera mandatory for general planetary exploration.

The characteristics of life, shape, growth, and movement can be caused by objects other than life. Therefore, it is important to combine the observations of shape, growth, and movement with verification by other methods, especially the information of the material constituting the structure.

4.4.4 Other Methods

Several other ways are also used to detect life features on Earth. The molecules deeply involved in the metabolic network (e.g., lipids, protein, DNA, and RNA) have been targeted for microbial cell detection. The procedure evaluating the amount of these metabolic molecules generally requires cell disruption, (partial) purification, and final determination of the concentration in the cell extract. The sensitivity of the final step depends on the following analytical methods: liquid absorption spectroscopy, many types of chromatography, and MS. The cellular component detection requires an extraction process consisting of many handling steps, which must be automated in the onboard machine. There is always the loss and the lower limit of handling samples, which uplift the overall detection limit. In some
cases involving special molecules (e.g., adenosine triphosphate (ATP)), they can be analyzed without these extraction processes and detected with significant sensitivity (Kamiloglu et al. 2020; Zhang et al. 2019).

The characteristics of extraterrestrial life are unknown; therefore, initiating considerations based on the characteristics of terrestrial life is rational in extraterrestrial life exploration. On the contrary, the probabilities of the analogy of each characteristic and the limit of the analogy must be considered along with the establishment of general methods beyond the analogy to life on Earth.

### 4.4.5 Combination of Life Detection Methods

Combining multiple methods is effective in concluding the existence of living things and grasping their characteristics (e.g., sterilizing half of the soil sample collected at one time, dividing them further and applying the proliferation method to half of the samples, and analyzing the samples with a fluorescence microscope). The samples can be investigated with a fluorescence microscope using various fluorescent pigments. The results will be then used for comparative studies. Section 5 describes the pigment combination.

Several reference observations have to be done in addition to the observations of the sample to be investigated: Observation of the sample-container without a sample, a sample at the surface irradiated by UV, and a sample before injection of the pigment solution. A combination of the above-mentioned method with molecular analysis like MS will be a powerful tool for clarifying the target characteristics: The general characteristics of the content targeted by pigment specificity, shape and size information will be obtained by the LDM, and the information on the molecular size will be obtained by MS.

### 5 Design of LDM and Usage on Mars and Other Places

This section describes the fluorescence microscope-based LDM system we developed for the exploration (detection and characterization) of extraterrestrial life and organic compounds. The fluorescence microscope is an instrument that is widely used in biology. This instrument irradiates a sample stained with a fluorescent pigment with excitation light. The fluorescence emitted from the pigment is then observed. While the design presented in this section is primarily intended for use in Mars, the LDM can be used for life exploration not only on Mars, but also on other places.

#### 5.1 Mars

**5.1.1 Fluorescence Pigments**

Among the fluorescent pigments detecting various biological components with respective specificity, pigments with the following characteristics were selected as the basic set for life exploration:

1. A pigment that detects organic compounds of both biogenic and abiogenic origin
2. A pigment that detects organic compounds surrounded by membranes
3. A pigment that detects a catalytic reaction
Earth organisms are composed of organic compounds. Organic compounds containing carbon atoms as the central atom connecting up to four reactive groups enable molecule polymerization and reactive residue rendering. Following hydrogen, helium, and oxygen, the carbon atom is the fourth most abundant atom in the universe (Lodders 2003). Therefore, carbon-based molecules should be the first targeted molecules to be searched outside Earth. Amino acids are found in meteorites and comets and have many reactive groups that can be used for catalytic reactions. Accordingly, amino acid polymers can be the catalytic molecules in possible life on Mars. SYPRO Red was used as a fluorescent pigment for the organic compound detection in (1) (Fig. 5). This pigment detects biogenic proteins, organic molecules, and nonbiogenic organic compounds, such as proteinoids (amino acid thermal polymers) that are thought to be produced in the prebiological process and polycyclic aromatic hydrocarbons (PAHs) contained in meteorites.

Earth organisms comprise a cell structure surrounded by a membrane that separates outside and inside and selectively permeates molecules and ions (Sect. 3.2). The membrane maintains the high concentration inside to enhance the catalytic reaction and keep the concentration difference across the membrane, which is responsible for signaling and producing energetic compounds like ATP. Such a membrane structure may be basic and common in Earth and extraterrestrial organisms.

Considering the above data, we plan to use a mixed pigment solution of SYTO24 and PI, which are two types of fluorescent pigments with different membrane permeabilities (Fig. 5),
to detect the membrane structure for (2). Hydrophilic PI cannot penetrate the cell membrane of living cells with an intact cell membrane structure. In contrast, SYTO24, which is highly hydrophobic, is permeable to intact and damaged cell membrane and stains organic compounds. The green fluorescence of SYTO24 is observed in living cells. Meanwhile, in dead cells with damaged cell membranes, PI permeates the cell membrane, and both PI and SYTO24 stain nucleic acids and other organic molecules. However, the green fluorescence of SYTO24 is absorbed by PI; hence, the red fluorescence from PI is dominantly observed.

The pigment for (3) detects catalytic reactions by enzymes supporting the metabolic network in cells. All Earth organisms utilize enzyme-catalyzed chemical reactions to sustain life activity (Sect. 3.2). We used the CFDA-AM fluorescent pigment that detects catalytic reactions by the most common enzyme, esterase (Fig. 5). CFDA-AM itself is nonfluorescent, but it emits green fluorescence when hydrolyzed by esterase in the cell. These pigments can stain normal cells and minicells that do not have DNA and have 1 µm or less diameter (Fig. 5) (Frazer and Curtiss 1975).

The fluorescent pigment solution contains 67% glycerol to cope with the low temperature and low pressure on Mars’ surface, making it possible to keep the pigment solution in a liquid state for the time required for staining and imaging at a temperature between $-30\degree C$ and $30\degree C$ and at 7 hPa atmospheric pressure on Mars. We tested and found that a pigment solution has sufficient resistance to perchloric acid, radiation, and temperature.

5.1.2 Sensitivity

One of the important strengths of the LDM is its high sensitivity. The sensitivity requirement is $10^4$ cells/(g soil), as the LDM goal. This value corresponds to two orders of magnitude higher than that in TV-GC/MS used in the Viking lander. This corresponds to the sensitivity at which microorganisms can be detected, even in places with the lowest microbial density on the ground on Earth. *Escherichia coli* contains approximately 0.3 pg of organic compound in a single cell, providing the detection limit of the organic compound by our LDM.

The upper limit of the density of Mars microorganisms can be obtained with the LDM, even if no microorganism is detected. The obtained results will be important for future manned explorations and planetary protection related to human safety.

5.1.3 Spatial Resolution

The spatial resolution requirement is 1 µm, as the LDM goal. The 1 µm value is close to the expected cell size and to the limit of the resolution inherited from the visible light wavelength. However, with the support of image analysis, it is basically possible to obtain some information on the microbial cell shape and size. This value was set based on the size of Earth prokaryotes (see Sect. 3.2). Earth was a world of prokaryotes for approximately 2 billion years after the birth of life. In contrast, Mars’ ocean and atmosphere were lost in 1 billion years. The characteristics of life on Mars are unknown; however, if an analogy from Earth is applied, the current Martian life corresponds to the prokaryotic stage.

Earth’s prokaryotes do not have organelles (e.g., nuclear, mitochondria) or intracellular transport machineries, and mass transfer within cells depends only on diffusion. Therefore, the cell diameter is unlikely to be larger than 1 µm, and the diameter of almost all prokaryotes (e.g., cocci or bacilli) is 1 µm or less.

SHERLOC in *Mars 2020* and RLS in *ExoMars* plan to visualize organic compounds. These instruments are based on detection by Raman spectroscopy, which is less sensitive
Table 3 Specifications for the LDM BBM

| Specification               | Description |
|----------------------------|-------------|
| Field of view              | 1.12 mm × 0.894 mm |
| Spatial resolution         | 1 µm (goal)  |
| Sensitivity                | $10^4$ cells/(g soil) (goal) |
| Excitation light source    | LD, $\lambda = 488$ nm |
| Lighting source            | LED + Koehler optics, $\lambda = 472, 525, \text{and} 632$ nm |
| Detector                   | CMOS, 1280 × 1024 pix, 8-bit |
| Number of sample chambers  | 20          |
| Mass                       | 5.7 kg total |
|                           | Fluorescence microscope: 1.8 kg |
|                           | Sample rotator: 1.6 kg |
|                           | Electronics unit: 2.3 kg |
| Power consumption          | 30 W        |
| Size                       | 160 mm × 120 mm × 240 H mm |
| Environment temperature    | $-30 \degree C \text{to} +30 \degree C$ (operation) |
|                           | $-40 \degree C \text{to} +70 \degree C$ (storage) |
| Environment pressure       | 7 hPa       |

This table is produced by modifying a table shown in the paper of Enya et al. (2021)

than fluorescence microscopy. The spatial resolution of these instruments is completely inadequate for observing the shape of 1 µm-sized targets. These features make the detailed investigation of 1 µm-sized microbial cells challenging.

5.1.4 Instrument Overview

We are developing a bread board model (BBM) to demonstrate the LDM. Table 3 and Fig. 6 present its specifications and appearance, respectively. The upper part of the instrument is a sample rotator containing 20 cylindrical sample chambers. Each sample chamber is equipped with two pigment-solution tanks. The soil collected by the robot arm of the Mars Rover is introduced to a sample chamber. The pigment solution flows from the tank into the sample chamber. The bottom of the sample chamber is a quartz window used to observe the sample from the bottom.

The lower part of the instrument is a fluorescence microscope with light sources. A blue laser diode (LD) with 488 nm wavelength is used as the excitation light source. The excitation light from the LD is incident on the lower part of the sample chamber through a dichroic mirror and objective lens. The fluorescence light emitted from the pigment passes through the objective lens, dichroic mirror, and imaging lens and reaches the image sensor.

In addition to the LD used as a fluorescence light source, light-emitting diodes (LEDs) are installed to enable imaging with normal lighting, helping observe the shape of mineral particles with a high resolution. Under the current specifications, the approximate dimensions and the target mass are 160 W × 120 L × 240 H (mm) and 5.7 kg, respectively. The power consumption is approximately 30 W. The operation and storage temperature ranges are $-30 \degree C \text{to} 30 \degree C$ and $-40 \degree C \text{to} 70 \degree C$, respectively. The fluorescence microscope in the LDM has the potential to reduce its mass. A further reduction of the total mass is possible if the number of sample chambers is reduced. The LDM specifications can be changed according to the various future plans of extraterrestrial life exploration.
5.1.5 False Positive

Mineral autofluorescence can be a false positive signal in extraterrestrial life exploration using a fluorescence microscope. We plan to use the difference in the fading rate and fluorescence spectrum to distinguish between mineral autofluorescence and microorganism fluorescence. Mineral autofluorescence hardly quenches when irradiated with excitation light, while fluorescence derived from pigments gradually quenches. Therefore, fluorescence images are taken before and after the excitation with irradiation for a certain period, and the obtained images are compared. A reduced fluorescence intensity after excitation light illumination corresponds to fluorescence derived from the pigment.

The comparison of the fluorescence of minerals and microorganisms potentially provides information for their identification because the peak wavelength and the half width are different from those of fluorescent pigments. Structural features, such as shape, size, and their uniformity, are also useful in overcoming the false positive.

Although completely eliminating false positives is hard, these methods can be used to minimize the number of particles detected by false positives.

5.1.6 Future Issues of the LDM

(1) Systematic survey of pigments The basic fluorescent pigments (i.e., SYPRO Red, SYTO 24, PI, CFDA-AM) were described. Many other fluorescent pigments stain the unique char-
acteristics of cells and organic compounds (Table 2). The target’s characteristics can be investigated in more detail. Furthermore, whether it is life or not can be judged based on various criteria using more fluorescent pigment types with different specificities. Therefore, we will be conducting a systematic survey to test more pigment types.

(2) Development with BBM of the LDM The BBM of the LDM was developed for use at a laboratory level (Table 3, Fig. 6). Using the BBM, we perform the development and demonstration of a staining solution addition mechanism in a low temperature and low pressure environment with the adjustment of the sample amount and the staining solution and a mechanism for opening and closing the sample chamber lid.

(3) Development of the Engineering Model of the LDM The major issues in the engineering model of the LDM are toughness against environment conditions, such as radiation, temperature, vibration, and humidity. Downsizing and lightweighting are also important. These technical hurdles will be cleared by applying the technical heritage in previous space flight equipment.

5.2 Returned Sample

Samples have been returned from space during the Stardust, Hayabusa, Tanpopo, and Hayabusa 2 missions. The analyses in the last two missions are still in progress. In the Stardust mission, aerogel blocks, which are blocks of amorphous silicate with low density, were used to collect impacted particles with hypervelocity. The returned aerogel blocks were inspected using an automated scanning microscope to identify the tracks formed by impacting particles. The aerogel keystones surrounding the tracks were extracted from the aerogel blocks and directly used for the detailed analyses or individual particle extraction (Westphal et al. 2004). The time-of-flight secondary ion mass spectrometry (TOF-SIMS) analysis of the Stardust sample showed the presence of PAHs that could possibly be attributed to Comet 81P/Wild 2 (Stephan et al. 2008).

The samples returned from Itokawa by Hayabusa were first analyzed by synchrotron radiation X-ray microcomputed tomography (MicroCT) to map the shape and the inner structure of dust particles in three dimensions (Tsuchiyama et al. 2011). The X-ray diffraction technique was then used to investigate the specific chemical composition of the minerals. In addition, computed tomography (CT) images were obtained with two different X-ray energies (i.e., 7 and 8 keV), enabling mineral identification and obtainment of the 3D distribution of each mineral in Spring 8, a synchrotron factory (Tsuchiyama et al. 2011). They concluded that Itokawa dust particles have a direct link between S-type asteroids and ordinary chondrites (Nakamura et al. 2011).

As described in Sect. 2, sample-return missions related to astrobiology have been planned. The MSR mission by NASA and ESA will bring samples back from Mars in the early 2030s (Muirhead et al. 2020). JAXA also plans a Phobos sample-return mission, called MMX. The MMX spacecraft will be launched in 2024 and return samples of the Phobos regolith back to Earth in 2029 (Kawakatsu et al. 2019; Usui et al. 2020).

Bringing samples back to Earth has several merits compared to sending a spacecraft for in situ exploration to Mars. The samples can be studied in laboratories using more advanced instruments with higher sensitivity, accuracy, and resolution of various analysis types. Multiple analyses are essential in conclusively identifying Martian samples. The particles detected by fluorescence microscopy indicates the highest sensitivity in detecting particles in a small amount of sample. The detected organic particles can be characterized by analysis.
Fig. 7  Aerogel developed for the Tanpopo project (presented in the study of Enya et al. (2021). The original photo is from Makoto Tabata). The inner part density is 10 mg/cm³ and reinforced with the outer part aerogel with 30 mg/cm³ density. It is manufactured with minimum contamination during production for utilization in the analysis of organic compounds and microorganisms.

Nanoscale secondary ion mass spectrometry (NanoSIMS) enables mapping of their constituent elements, such as C, N, and O, including their isotopes (Suzuki et al. 2020). Samples can be analyzed by scanning transmission X-ray microscopy/X-ray absorption near edge structure (STXM-XANES) (Kebukawa et al. 2019; Yabuta et al. 2014) to further analyze the bonding type of a specific atom. SEM and TEM allow cell-like structures to be revealed in detail, with energy-dispersive X-ray spectroscopy (EDX) for analyzing the distribution of constituting atoms. Liquid or GC with MS with an ion-accumulating ability (e.g., Orbitrap (Thermo Fisher Scientific)) is effective in identifying organic compounds (e.g., amino acids, lipids, polysaccharides, and fatty acids), which are essential components for terrestrial organisms, with the sensitivity of tens of molecules. DNA analysis by massive parallel sequencing is also useful in checking for contamination of Earth organisms.

The number and type of amino acids are the key indicators, as mentioned in Sect. 3.1.3. The return of samples containing living organisms would significantly contribute to the study of the origin and evolution of life considering that amino acid chirality is likely to be better preserved in living organisms than past organisms. In addition to the initial investigation of the returned samples, the LDM for detecting the biosignatures of living organisms is also a powerful tool for selecting samples that are worth bringing back to Earth.

5.3 Others

5.3.1 Icy Bodies

The various icy bodies in the solar system have large amounts of H₂O. Icy moons, such as Jupiter’s moons Europa, Ganymede, and Callisto, and Saturn’s moon Enceladus are especially interesting. These icy moons have a subsurface ocean, whose primary composition is liquid water. Their surfaces are composed of ice crust in these solar system bodies (Kivelson et al. 2002; Schubert et al. 2004; Thomas et al. 2016). Plumes have been observed in Europa and Enceladus (Porco et al. 2006; Roth et al. 2014). The environments related to these liquid subsurface oceans are potential sites for life exploration.

Aerogel is a candidate material for the plume sampler. Sampling by aerogel was the method used in the Tanpopo project (Yamagishi et al. 2014) (Fig. 7). A much larger amount of solid sample can be obtained if the spacecraft can directly land on an icy moon and collect ice from its surface. Having access to the subsurface ocean would be very valuable; however,
breaking through the thick ice crust will be highly challenging. For icy objects, both in situ experiments and sample returns are beneficial for life exploration.

5.3.2 Venus

The 48–70 km-high Venus cloud layer is a potential habitable zone (Cockell 1999) composed of sulfuric acid-based aerosols. This atmospheric aerosol may contain microorganisms (Limaye et al. 2018); therefore, the LDM to be mounted on the aerial platform is being studied to investigate the microorganisms and organic compounds in the aerosols of the Venusian atmosphere (Sasaki et al. 2022). There is a plan to use an impactor to perform sampling in Venus missions. In situ experiments are envisioned for life exploration in the atmosphere of Venus, which is currently under consideration. Sample returns are also considered worthwhile.

5.3.3 Asteroids and Martian Moons

Japan is currently conducting a series of sample-return missions. Hayabusa brought back a sample of the Itokawa asteroid in 2010 (Kawaguchi et al. 1996; Tsuchiyama et al. 2011; Yoshikawa et al. 2015). Hayabusa 2 succeeded in sampling on the Ryugu asteroid and already returned to Earth (Morota et al. 2020; Tsuda et al. 2020). Following these, the development of MMX, which aims to bring back a sample of the Martian moon, Phobos, is underway (Kawakatsu et al. 2019; Usui et al. 2020). Section 2.6 describes more relevant details.

Life exploration for small bodies, especially the investigation of Mars-derived dead microorganisms in Phobos, is important in terms of the Panspermia hypothesis that assumes the microorganism transferred in space. The LDM is not installed on these kinds of spacecraft. The amount of the return sample is small and extremely precious; hence, whether or not the LDM is finally allowed in the program for the returned sample analysis remains a question. However, we believe that the returned sample is worth analyzing with the LDM for the scientific goal because the sample required for the LDM is only approximately 10 mg.

5.3.4 Moon

It looks reasonable to consider that the possibility of the moon having its own life is lower than that of Mars due to the harsher environment. In contrast, microorganisms can possibly be transferred from Earth and reach the moon because the moon is close to Earth (Yang et al. 2009). The analysis of the microorganisms on the moon surface will give us direct and, possibly the first, information of the Panspermia hypothesis. Therefore, we are considering the LDM application for moon in an in situ analysis and/or analysis for the returned samples.

6 Summary

In this paper, we reviewed the exploration of extraterrestrial life signatures in the solar system, focusing on fluorescence microscopy. Mars is a promising place to search for signatures of life using a fluorescence microscope, which can also be applied to Venus’ clouds, moon, asteroids, and icy bodies (e.g., ice moons of Jupiter and Saturn). Considering the targeted characteristics similar to those of terrestrial microorganisms is a useful strategy: microorganisms with uniform spherical or rod structures that are approximately 1 µm in diameter.
are surrounded by a membrane with metabolic activity and made of carbon-based molecules. These characteristics can be analyzed by using a fluorescence microscope with a combination of fluorescence pigments and staining specificity to distinguish the characteristics of life.

Following the introduction in Sect. 1, past and present Mars exploration missions were summarized in Sect. 2 based on their employed science instruments, methods, and achievements closely related to astrobiology. The described missions were Viking, MSL, Mars Express, ExoMars, MARS 2020/MSR, and MMX. Section 3 described the targets of astrobiological search and analysis. Various kinds of organic compounds, including the following, became important targets in Mars exploration: (1) organic compounds biologically formed on Mars; (2) those carried from space to the surface of Mars; (3) those biologically formed by past life; and (4) those biologically formed by the extant life of Mars. Catalysis, molecules, and structures were discussed as targets of astrobiological search and analysis for microorganisms. Section 4 reviewed how to detect and characterize extraterrestrial life and organic molecules. We described the microscopy and MS together with other methods (based on mass spectrometry, morphology, detection of growth, movement, and death, etc. for microscopic and macroscopic). Combinations thereof were also discussed. A fluorescence microscope showed high sensitivity and spatial resolution and can obtain a high-contrast image of the astrobiology target itself. The biological and chemical characteristics of the targets can also be estimated using fluorescent pigments with various binding specificities. Therefore, the fluorescence microscope is important and complementary to the mass spectrometer, which detects molecules related to extraterrestrial life. Section 5 introduced the LDM we developed for the detection and characterization of the extraterrestrial life on Mars and other sites. The manufacturing of the BBM of the LDM has been completed, and tests are ongoing.

In situ measurement and detailed analyses of the samples brought back by sample-return missions are complementary in the extraterrestrial life exploration in the solar system. Fluorescence microscopy is a promising candidate instrument for both purposes.

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