Chapter 23
The Search for Life on Mars

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Abstract Though Mars is a cold and dry planet now, Mars would have harbored a large amount of liquid water on the surface early in its history. Mars could have been similar to the early Earth from which life arose 4 billion years ago, and life may have also emerged on Mars during this period. Although the Viking mission in 1976, which explored life on Mars, did not find evidence for life, many findings associated with the possibility of life have been discovered since the Viking mission: past and present aqueous environments, organic compounds, methane, reduced compounds suitable for microorganism energy sources, and so on. These findings suggest that life might exist on Mars. Habitable environments may be deep subsurface, but it may also be on or near the surface where physical and chemical conditions on which even terrestrial microorganisms to survive are found. Life detection instruments have been developed since the Viking mission. Traces or existence of Martian life might be found by future exploration.

Keywords Habitability · Organic compounds · Living microorganisms · Chemolithoautotrophs · Life detection instruments

23.1 Introduction

The possibility of life on Mars has enamored many people for many years. In the 1970s, the Viking landers conducted search-for-life experiments and failed to detect evidence of life on the planet. After the Viking mission, both the National Aeronautics and Space Administration (NASA) and the European Space Agency (ESA) focused on investigating ancient habitability in their Mars exploration programs. Evidence of past liquid water activities has been reported: large outflow channels found by the Mars Global Surveyor (Malin and Carr 1999; Malin and Edgett 2000), H_2O ice under tens of centimeters of soil found by the Mars Odyssey Neutron Spectrometer (Feldman et al. 2002), hydrated sulfate and phyllosilicates found by the Mars

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Express spacecraft (Gendrin et al. 2005), sedimentary rocks found by the Mars Exploration Rover Opportunity (Squyres and Knoll 2005), and an H_2O ice table found by the Phoenix lander (Smith et al. 2009). These findings suggest that ancient Mars sustained large quantities of liquid water and contained suitable conditions for life (see Chap. 21; Lasue et al. 2013). Assuming that life emerges in suitable conditions, it can be hypothesized that, since life arose within a few hundred million years after the formation of Earth's surface (McKay and Davis 1991), life could have also arisen on early Mars.

Even on Mars today, locations indicating the possible presence of liquid water have been found. Furthermore, organic compounds and energy sources used by terrestrial microorganisms have been discovered on the surface. This chapter reviews the possibility of life on Mars and the potential for exploration.

23.2 The Viking Mission and Recent Reexaminations

NASA conducted life exploration experiments with the Viking mission in 1976; however, the existence of life could not be verified from the surface soil samples (Klein 1977, 1978, 1979, 1992; Margulis et al. 1979). The Viking landers carried out three biological experiments on the Mars surface: the pyrolytic release (PR) experiment for detecting carbon assimilation, the labeled release (LR) experiment for detecting the decomposition of organic compounds, and the gas exchange (GEX) experiment for detecting changes in gas composition caused by metabolic reactions (Fig. 23.1).

In the PR experiment, carbon dioxide and carbon monoxide labeled with radioactive carbon-14 (^{14}C) were added to the soil sample with water and irradiated with light. In the presence of organisms, ^{14}C is incorporated, and organic compounds are produced. These compounds were pyrolyzed, and the released ^{14}C was measured with a radiation detector. Although small amounts of CO_2/CO were incorporated, this incorporation was considered non-biological, because similar levels of incorporation were seen after heating the samples at 90 °C for 2 h.

In the GEX experiment, a nutrient medium containing organic substances, such as amino acids and vitamins, and a mixed gas composed of CO_2 and Kr (in He) were added to the sample chamber. The changes in the gas composition were analyzed by gas chromatography after several days. If organisms were present, the gas composition would be changed by the release of carbon dioxide. Although the CO_2 evolution was observed, it was thought to have come from the oxidation of organics in the nutrient medium by indigenous oxidants like Fe_2O_3 (Oyama and Berdahl 1977).

The LR experiment was conducted by adding seven liquid nutrients (formate, glycolate, glycine, D-alanine, L-alanine, D-lactate, and L-lactate) labeled with ^{14}C, to the samples. The release of radioactive carbon (such as $^{14}CO_2$) was expected, if organisms metabolized the nutrients. The results showed positive responses that were consistent with biological activities: radioactive gas was evolved, and the gas evolution was reduced or not observed when samples were heated at 46 °C or

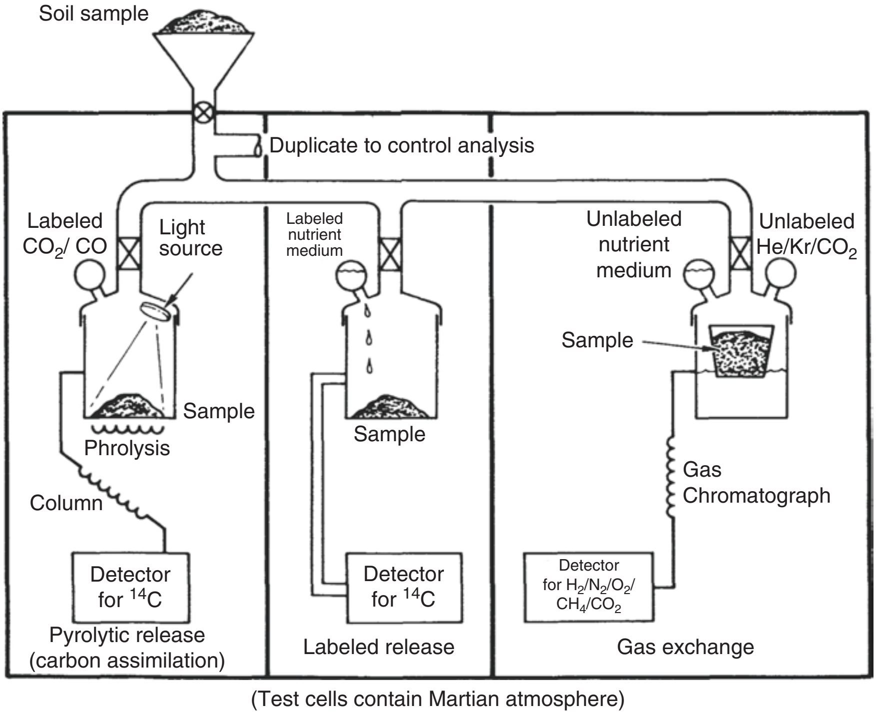

Fig. 23.1 Schematic diagram of the three biological experiments conducted by the Viking landers (Adapted from Viking 1 Early Results, NASA SP-408, 1976)

160 °C, respectively (Levin and Straat 1977). However, the results were still interpreted to be non-biological responses. This interpretation was based on the following results: (1) organic compounds were not detected at levels above the detection limit of the thermal volatilization–gas chromatography–mass spectrometry (TV-GCMS) instrument, and, although chlorinated organics (chloromethane and dichloromethane) were detected, they were interpreted as terrestrial contamination (Biemann et al. 1977); (2) the results could be explained by the presence of oxidants in the regolith (Klein 1978; Margulis et al. 1979). Therefore, the most acceptable conclusion of the Viking experiments was that no organisms were present within the detection limits of these experiments (Klein 1977, 1998, 1999).

However, after the Viking mission, instrumental limitations were reported. The Viking TV-GCMS was not specifically designed to identify the presence of living cells, and the pyrolysis products of cells would not have been detected if living cells were present in quantities less than 10^7 cells per gram (Glavin et al. 2001). Nonvolatile salts of organic acids and low levels of organic compounds would not have been easily detected by the TV-GCMS (Benner et al. 2000; Navarro-Gonzalez et al. 2006). Thus, the existence of organic compounds on Mars could not be accurately determined by the Viking instrument.

23.3 Habitability

In the years since the Viking mission, both NASA and ESA have looked for evidence of ancient habitability, such as traces of past water activities. An ancient possible habitable environment was discovered by the Curiosity Rover at Yellowknife Bay in Gale Crater (Grotzinger et al. 2014). The site was determined to be an ancient lake, with neutral pH and low salinity. Reduced iron and sulfur, as possible microbial energy sources, as well as biogenic elements (C, H, O, S, N, P), have also been detected. On modern-day Mars, life (most likely microorganisms) might exist at least locally since organic compounds, possible liquid water, and energy sources have been found. Figure 23.2 provides a schematic drawing of the possible habitability of present-day Mars. To learn more about both past and present habitability on Mars, see a review by Cockell (2014).

23.3.1 Organic Compounds

If life existed on Mars, organic compounds would be present. The detection and interpretation of organic compounds on Mars are complicated by the existence of perchlorates. While the Viking TV-GCMS did not identify any organic compounds, the Phoenix lander conducted a chemical analysis and detected the presence of 0.4–0.6 wt% perchlorate anion (ClO_4^-) from the surface soil (Hecht et al. 2009). Perchlorate is rare on the surface of Earth; it occurs naturally in hyperarid environments, such as

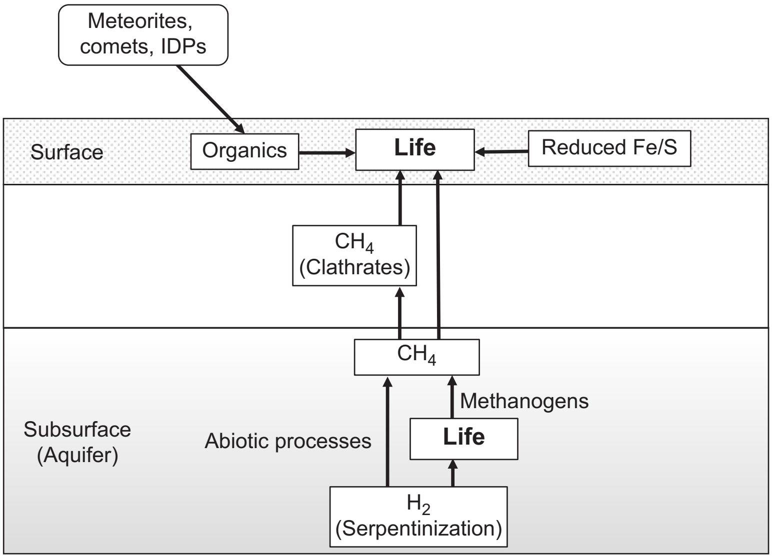

Fig. 23.2 Schematic diagram of possible habitability on modern-day Mars

the Atacama Desert in Chile (Catling et al. 2010) and the Antarctic Dry Valleys (Kounaves et al. 2010). Though perchlorate salts are stable at low temperature, they become strong oxidants when heated, decomposing organic compounds.

Recently, the Curiosity Rover detected chlorinated compounds (chlorobenzene and C2 to C4 dichloroalkanes) (Ming et al. 2014; Freissinet et al. 2015) and thiophenic, aromatic, and aliphatic compounds (Eigenbrode et al. 2018) in the mudstones in Gale Crater. The chlorinated compounds were interpreted to be the reaction products of pyrolysis between oxychlorine compounds, such as perchlorate, and indigenous organic compounds (Freissinet et al. 2015). However, identification of the original organic compounds is difficult due to the complex reactions during pyrolysis. Thus, it is uncertain whether these compounds are derived from Martian sources (igneous, hydrothermal, atmospheric, or biological) or exogenous sources (meteorites, comets, or interplanetary dust particles (IDPs)) (Freissinet et al. 2015). Due to the discovery of perchlorates, it has been noted that the chlorinated compound found by the Viking TV-GCMS might also be the reaction product of indigenous organic compounds and perchlorates during pyrolysis of the soil samples at 500 °C (Steininger et al. 2012; Lasne et al. 2016). Although the discovery of organic compounds does not indicate evidence of life, it also does not rule out the possibility of life. The identification and characterization of organic compounds will be important for future Mars missions.

23.3.2 Liquid Water

Liquid water is a fundamental requirement for life. As described in Chap. 21, ancient Mars had a large amount of liquid water on the surface, but liquid water is unstable on the present Martian surface because of low temperatures and pressures. Water on the surface exists mainly in the form of ice, which organisms cannot use. Ground ice has been identified near the surface of the planet by orbiting neutron detectors on the gamma-ray spectrometer carried by Mars Odyssey (Feldman et al. 2002), and the Phoenix lander has shown a shallow H_2O ice table at depths of 5–18 cm in the northern arctic region (Smith et al. 2009).

The possibility of liquid water has also been reported on the Martian surface. For example, recurring slope lineae (RSL), narrow dark streaks on steep slopes that appear during warm seasons in equatorial regions, could be a result of liquid water flow (McEwen et al. 2014) as hydrated salts of magnesium perchlorate, magnesium chlorate, and sodium perchlorate were observed at some of the flows sites (Ojha et al. 2015), although, as another interpretation, it could be dry granular flows of sand and dust (Dundas et al. 2017). The salt solutions can lower the freezing point and the evaporation rate of water. For example, highly concentrated perchlorate solutions remain at liquid state below about −70 °C (Möhlmann and Thomsen 2011). In addition to RSL, the Curiosity Rover demonstrated that transit liquid brine is formed at night in the shallow subsurface at Gale Crater, based on the meteorological analysis. Recently the Mars Advanced Radar for Subsurface and Ionosphere Sounding (MARSIS) instrument on the Mars Express spacecraft has detected the evidence of stable liquid water, possibly perchlorate brines, about 1.5 km below the surface at the southern polar ice cap (Orosei et al. 2018). Liquid brines could be abundant on Mars, since perchlorates are widespread on the surface (Martín-Torres et al. 2015). Although it is uncertain whether the Martian brines have sufficient water activity to support life (Martín-Torres et al. 2015; Edwards and Piqueux 2016), microbial growth on Earth is known to occur in highly concentrated salt solutions (Grant 2004), and some halophilic microorganisms can use perchlorate as an electron acceptor for respiration in anaerobic conditions (Oren et al. 2014). It can be inferred, therefore, that microorganisms may survive in the briny environments on Mars.

23.3.3 Energy Sources

Life requires energy for its growth, reproduction, and survival. Terrestrial organisms that obtain chemical energy from the oxidation of reduced compounds (energy sources) are named *chemotrophs*, while organisms that use light as an energy source are named *phototrophs*. Chemotrophs are further classified into chemoheterotrophs, which use organic compounds as energy sources, and chemolithoautotrophs, which use inorganic compounds as energy sources. Electron transport from reduced compounds (electron donors) to oxidative compounds (electron acceptors) generates energy for the organisms, which can be calculated as Gibbs free energy. There are many combinations of electron donors and electron acceptors in terrestrial microorganisms, some of which may be used by Martian microorganisms.

Table 23.1 provides examples of potential energy sources for chemotrophs on Mars, which are known to exist or are strongly inferred to exist on the planet. The metabolism of these sources, including H_2, CH_4, S^0, S^{2-}, Fe^{2+}, CO, and organic compounds, has been confirmed in terrestrial microorganisms (Cockell 2014; Rummel et al. 2014; Westall et al. 2015; Cockell et al. 2016). Potential electron acceptors in

Table 23.1 Examples of potential energy sources for chemotrophic life on Mars (Adapted from Rummel et al. 2014; Cockell 2014; Westall et al. 2015)

Energy sources	Electron acceptor	Metabolism
H_2	CO_2	Methanogenesis, acetogenesis
H_2	Fe^{3+}, SO_4^{2-}, S^0, ClO_4^-	Hydrogen oxidation
CH_4	NO_3^-, Fe^{3+}, MnO_2, SO_4^{2-}	Methane oxidation
S^0, S^{2-}	NO_3^-, Fe^{3+}, MnO_2	Sulfur oxidation
Fe^{2+}	NO_3^-, MnO_2	Iron oxidation
CO	CO_2,	Methanogenesis, acetogenesis
CO	NO_3^-, H_2O, SO_4^{2-}, ClO_4^-	Carbon monoxide oxidation
Organics	NO_3^-, Fe^{3+}, SO_4^{2-}, ClO_4^-	Anaerobic organics oxidation
Organics	Organics	Fermentation

These substances are known to exist or are strongly inferred to exist on Mars. The redox couples have been confirmed in terrestrial microorganisms anaerobic conditions are CO_2, Fe^{3+}, SO_4^{2-}, S^0, NO_3^-, MnO_2, ClO_4^-, H_2O, and organic compounds. Oxygen that is produced by photolysis/radiolysis of water might be used as an electron acceptor, although the amount of O_2 is much lower in the Martian atmosphere than on Earth (Westall et al. 2015). Low concentration of molecular hydrogen (H_2) was detected in the upper atmosphere by the space-based telescopes from Earth, which was likely produced by photolysis of water vapor (Krasnopolsky and Feldman 2001). Although H_2 has not been directly measured on Mars surface yet, it is inferred from the presence of olivine and serpentine (Oze and Sharma 2005; Schulte et al. 2006). Fe-bearing minerals and elemental sulfur have been identified on Mars surface (Morris et al. 2007), as well as reduced sulfur such as sulfides (Ming et al. 2014). Nitrate has not been directly detected yet, but detection of NO by the Curiosity Rover suggests the possible presence of nitrate (Stern et al. 2015). Indigenous organics may also be energy sources for chemoheterotrophs, although their structures and accessibility are unknown.

Among these energy sources, methane is a molecule with special interest, because it can be an energy source for methane-oxidizing microorganisms (*methanotrophs*). Methane generation is associated with microbial activities on Earth, where around 80% of natural emissions of methane originate from living microorganisms (Etiope et al. 2011).

Methane in the Martian atmosphere has been reported using a variety of methods (see Chap. 22): Earth-based telescopic observations (Krasnopolsky et al. 1997; Krasnopolsky et al. 2004; Mumma et al. 2009), the Planetary Fourier Spectrometer on board the ESA Mars Express (Formisano et al. 2004; Geminale et al. 2011) ranging from several to tens of parts per billion by volume (ppbv), and the tunable laser spectrometer in the Sample Analysis at Mars on the Curiosity Rover at ~7.2 ppbv (Webster et al. 2015). Spatial and seasonal variations of methane (Mumma et al. 2009; Webster et al. 2018), combined with its relatively short lifetime in the Martian atmosphere of about 300 years (Krasnopolsky et al. 2004) and potentially less than 200 days (Lefevre and Forget 2009), indicate that methane has been released into the atmosphere locally and periodically. Though the origins of this methane are uncertain, several generation processes have been proposed, including biotic (microbial) and abiotic processes.

Biotic methane is produced by microorganisms called methanogens, which are anaerobic ones belong to the domain Archaea. Most methanogens use H_2 as an energy source and CO_2 for a carbon source, and some methanogens use CO, acetate, methanol, etc. as energy sources. An important H_2 origin could be subsurface serpentinization (Atreya et al. 2007). Serpentinization is a reaction of olivine- and pyroxene-rich rocks with liquid water, liberating H_2 in the process (Schulte et al. 2006). Carbon dioxide may be derived from the atmosphere, magma degassing, or the thermal decomposition of carbonates on Mars (Oehler and Etiope 2017).

Hydrogen is used as an energy source not only for methanogens but also for a wide variety of chemolithoautotrophic microorganisms on Earth (Table 23.1). Since higher temperatures and pressures would sustain liquid water stably at depths below a few kilometers, a microbial community may exist in the Martian subsurface (Chapelle et al. 2002; Clifford et al. 2010; Michalski et al. 2013).

Possible abiotic methane production mechanisms include geological productions like hydrogeochemical Fischer-Tropsch-type (FTT) reactions after serpentinization (Oze and Sharma 2005); thermogenesis of organics delivered to Mars by meteorites, IDPs, or possible biotic organics (Etiope et al. 2011); geothermal reactions at high temperatures (Oehler and Etiope 2017); ultraviolet degradation of meteoritic organics (Keppler et al. 2012); production by the impact of comets (Krasnopolsky 2006); and volcanic degassing (Atreya et al. 2007). Among them, FTT reactions, which produce methane from the reaction of H_2 with CO_2, could be important, since they are major abiotic producers of methane on Earth that occur over a wide range of temperatures (<100 to ~500 °C) (Etiope and Sherwood Lollar 2013).

Biotic or abiotic methane could be released into the atmosphere directly and/or via *clathrates* (methane-hydrates) or gas-absorbing regolith. Thus, the presence of methane today does not require the presence of living methanogens; it may have been produced by past methanogens and preserved (Max and Clifford 2000; Atreya et al. 2007).

23.3.4 Physical and Chemical Conditions

Terrestrial microorganisms inhabit a wide range of environmental conditions (see Chap. 20). Although present Martian environments are hostile to life, some microorganisms may survive near the surface (Yamagishi et al. 2010). Microorganisms isolated from a Siberian permafrost sample, for example, were capable of growth under simulated Mars conditions: low temperature (0 °C), low pressure (7 hPa), and an anoxic CO_2-dominated atmosphere (Nicholson et al. 2013).

Radiation would be a serious limiting factor for microbial survivability, since organic compounds are likely to be destroyed by ionizing radiation and UV radiation (Benner et al. 2000; Kminek and Bada 2006). The total dose of ionizing radiation on the Martian surface was measured as 76 mGy/year by the Curiosity Rover (Hassler et al. 2013). However, a radiation-tolerant microbe, *Deinococcus radiodurans,* can survive 5 kGy without loss of viability (Cox and Battista 2005; Dartnell et al. 2007); thus, ionizing radiation would not seriously damage these microorganisms. Though UV radiation is harmful, it would be shielded by thin layers (less than a millimeter) of dust or regolith (Mancinelli and Klovstad 2000). A depth of several centimeters from the surface, therefore, could provide sufficient covering for microorganisms to survive.

23.4 Life Detection Instruments and Possible Explorations

There are many biosignatures for the targets of life explorations, including organic compounds, metabolic activities, cell-like morphology, and stable isotope patterns. Organic compounds are important targets, and the GCMS is an effective instrument for the detection of organic compounds. However, as mentioned earlier in this chapter, analyses by the TV-GCMS on Mars were affected by perchlorates, which react with indigenous organics during pyrolysis. In forthcoming missions, including the Mars 2020 mission and the ExoMars 2020 mission, instruments designed to detect organic compounds without pyrolysis have been selected.

The Mars 2020 rover will detect organic compounds with the Scanning Habitable Environments with Raman and Luminescence for Organics and Chemicals (SHERLOC), which will detect and characterize minerals and organic compounds, such as aromatic hydrocarbons, with a resonance Raman spectrometer and a fluorescence spectrometer that utilizes a deep-UV laser (<250 nm) (Abbey et al. 2017). It has a context imager with a spatial resolution of 30 μm to visualize surface textures, morphology, and visible features correlated with the spectral signatures (Beegle et al. 2015). The rover will also select and cache the highest value samples for a future sample-return mission, which will take the samples to laboratories on Earth for advanced analysis.

The ExoMars 2020 rover will be equipped with a drill to collect materials from outcrops at depths down to 2 m. The organic compounds will be detected by the Mars Organic Molecule Analyzer (MOMA), which includes two different types of analysis methods, laser desorption mass spectrometry (LD-MS) and TV-GCMS, with or without derivatization agents (Vago et al. 2017). The LD-MS method is not affected by perchlorates, and the derivatization process will be useful for detecting refractory molecules like carboxylic and amino acids. It will be also equipped with a Raman laser spectrometer that will identify minerals and organic compounds (Vago et al. 2017).

The candidates for landing sites in both the Mars 2020 and ExoMars 2020 missions are places with evidence of past water activities (Ono et al. 2016; Kereszturi et al. 2016). Although RSL where possible liquid water/brine exists could indicate attractive sites at which to search for extant life, RSL are not considered indicators for high-priority sites for either project. Both missions have mainly focused on investigating *ancient* habitability; additionally, explorations of RSL would include Committee on Space Research (COSPAR) Planetary Protection constraints to protect Mars from contamination from terrestrial organisms (Rummel and Conley 2017). The current COSPAR Planetary Protection Policy defines Mars special regions as locations in which Earth life could propagate, where the temperature is at or above −28 °C and water activity is at least 0.5 (Kminek and Rummel 2015; Rummel and Conley 2017). Although no confirmed special regions have been shown on Mars, RSL indicate possible candidates (Rummel et al. 2014; Rummel and Conley 2017). Exhaustive discussion will be required for explorations in RSL areas.

Other attractive sites where to search for present life include methane seepage sites. Even though methanogens and other microorganisms may exist in the deep subsurface, it is difficult explore those areas. Methanotrophs, however, may be found near the surface (Yamagishi et al. 2010). Some methanotrophs on Earth utilize MnO_2, $Fe(OH)_3$, and SO_4^{2-} as electron acceptors (Beal et al. 2009), all of which have been found on the Martian surface. Potential methane seepage sites have been indicated on Mars, such as mud volcano-like mounds, ancient springs, and rims of large impact craters (Oehler and Etiope 2017). When future work by the Trace Gas

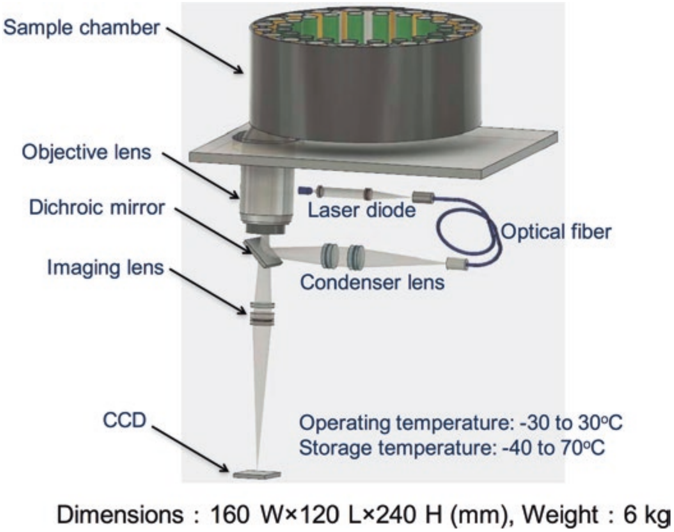

Fig. 23.3 Conceptual design of LDM (Life Detection Microscope) (Adapted from Yamagishi et al. 2018 (© 2018 by the Japan Society for Aeronautical and Space Sciences and ISTS))

Orbiter or surface rovers determines the locations of methane seepage sites, those sites could become candidates for future exploration.

A microscopic instrument would be a powerful tool for searching for extant microorganisms, but it has not been used in space missions yet (Nadeau et al. 2008; Yamagishi et al. 2010). Microscopes directly image life forms and identify their shapes, sizes, and other morphological structures. The Life Detection Microscope (Fig. 23.3) proposed by Yamagishi et al. (2018) detects organic compounds at a spatial resolution of 1 μm, differentiating among organic compounds surrounded by membranes or with enzyme activity by staining the samples with fluorescent pigments. This technique is especially useful for the detection of living microorganisms.

The search for living microorganisms is important not only for scientific interest but for planetary protection. Before future human missions begin, surveys investigating the presence of living microorganisms should be conducted to mitigate the risk of human contact with Martian microorganisms, which may be harmful to human health. Microscopic instruments would be effective tools for this purpose.

23.5 Conclusions

Although the Viking mission failed to detect Martian life, recent findings, such as the presence of organic compounds, energy sources, and possible liquid water, have suggested the possibility of life near the planet's surface. Life detection instruments have been developed since the Viking mission that use Raman spectrometry and

LD-MS without pyrolysis to avoid the problem caused by the reaction between indigenous organic compounds and perchlorates. Microscopic instruments that are particularly superior for detecting living microorganisms have also been proposed. These in situ instruments and a future sample-return mission might reveal the existence of life on Mars.

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