LIFE DETECTION MICROSCOPE FOR IN-SITU IMAGING OF LIVING CELLS ON MARS. A. Yamagishi (Tokyo University of Pharmacy and Life Sciences/JAXA; yamagish@toyaku.ac.jp), H. Demura (Aizu University), K. Fujita (JAXA), H. Honda (Nagaoka Institute of Technology), E. Imai (Nagaoka Institute of Technology), A. Miyakawa (Tokyo University of Pharmacy and Life Sciences), H. Miyamoto (University of Tokyo), S. Ohno (Chiba Institute of Technology), T. Ozawa (Aerospace Research and Development Directorate /JAXA), S. Sasaki (TEU, S. Sasaki (Osaka U), T. Satoh (ISAS/JAXA), Y. Yoshimura (Tamagawa University), and T. Usui (TITECH)

Introduction: Past trial of direct detection of life on Mars by the 1970's Viking mission resulted in a negative conclusion [e.g., 1], whereas numbers of circumstances provided by recent exploration missions in the last decade indicate that there are good reasons to perform another life detection program [e.g., 2 and reference therein].

Recent explorations have provided evidence for (1) the existence of large bodies of ancient surface water (paleo-ocean/lakes) [e.g., 3, 4, 5], (2) a limited but certain period of wet and warm paleo-climate probably suitable for life [e.g., 6, 7], and (3) the existence of strong magnetic field that could have protected the atmosphere from being quickly eroded away in the earliest stage of the Mars history [e.g., 8]. These would lead one to argue that the surface environment (e.g., hydrologic activity) in ancient Mars was not very different from that for Earth. This argument is strongly supported by results from the Curiosity rover [e.g., 9, 10, 11].

Even present Mars seems to retain environment supportable primitive life. Traces of possible liquid water flow have been reported at a number of locations including those recognized as the recurring slope lineae, seasonal flows on slopes of several craters [e.g., 12, 13]. These features as well as others proposed to be formed by running water flows suggest that subsurface brine could persist longer period providing a habitable environment. In addition, the SAM (Sample Analysis on Mars) carried by Curiosity recently detected hydrogen sulfide released from a Martian regolith sample when heating to the sample was applied (Leshin et al. 2013). The result indicates the presence of reductive compound in the Martian soil. The reductive element combined with oxidative compound such as perchlorate which is ubiquitous in Martian regolith offers a variety in oxidation states. This can be the energy source for chemolithoautotrophic (at lease for terrestrial) microbes.

Recent fast progress in our knowledge of microbes in terrestrial extreme environments, called extremophiles, has been changing our idea about life. There are some terrestrial microbes capable of surviving and even proliferating under the operation of each of the tough environmental factors expected at Mars: Most terrestrial organism is able to proliferate as long as the maximum surface temperature reaches above 0ºC. A microbe strain capable of replicating under a low atmospheric pressure (0.7% of terrestrial surface pressure) was found. The Martian surface radiation dose rate of 150 mGy per year is far below what kills radiation-tolerant microbes Deinococcus species. UV light can be enough shielded by several centimeters of regolith. These lines of arguments lead one to argue that terrestrial-type microbe may survive at several centimeters below the surface of Mars. Life has ability to adapt and then to survive in the course of evolution. Martian life would adapt to the Martian environment once it emerged.

In judging between the negative conclusion by Viking and the recent encouraging findings, one should wonder if the Viking onboard instrument was sensitive enough to judge possible existence of life on the severe environment of Mars. For this reason, we develop Life Detection Microscope (LDM) as a possible instrument onboard future Mars mission, that has much higher sensitivity than the instrument onboard Viking.

Life Detection Microscope: The NASA pioneering mission Viking Probe, equipped with Thermal-volatilization Gas-chromatography-Mas-spectrometer (TV-GC-MS) analyzed Martian soil and found that the content of organic compound was under the detection limit. Reanalysis of the detection limit of the TV-GC-MS shows that more than 10^7 cells per 1 g Martian regolith is needed for positive detection. This lower limit, however, means that the instrument could not detect any life even at Puna de Atacama, one of the driest places on Earth. In this toughest environment on Earth, only 10^4 bacteria per 1g of clay exist. An instrument to detect life elsewhere than Earth had better have the sensitivity that allows it to conclude positive detection at the toughest place on Earth. Our new instrument (LDM) is designed to detect less than 10^4 cells in 1gram clay. In other words, LDM has the sensitivity that is three orders of magnitude higher than the one onboard Viking that issued the negative conclusion.

LDM is designed to search for possible "cells" in the regolith at several centimeters below the surface of Mars. Equipped with 1 μm/pixel resolution, which is

more than ten times higher than any microscopic imager flown in space, LDM will be able to get high-resolution visible images of regolith and dust particles. LDM is also equipped with specially designed pigment system to distinguish biotic organic compounds from abiotic. The system is also designed to highlight biotic organic compounds surrounded by membrane.

Our strategy for detecting life is to detect organic compounds that have the characteristics feature seen in terrestrial life. Terrestrial life utilizes organic compounds to sustain itself. The compounds should be separated by an envelope from its environment so that variations in the external conditions would less dramatically affect their function. It is in this stabilized environment that the organic compounds possess the catalytic activities for generating free energy for life to survive. While we do not know how life on Mars works, we do know that organic compounds surrounded by membrane ("cells") are the fundamental framework for terrestrial life. As far as we know, all the terrestrial life has cells as the building block. It would be reasonable to consider it to be the fundamental framework for life on Mars as well. What follows from this consideration is that a life detection instrument should be able not only to detect organic compounds but also to characterize them from this perspective. LDM is capable of detecting and characterizing organic compounds by using a combination of fluorescent dyes.

We has successfully identified the combination of fluorescent dyes that enables (1) to detect organic compounds including those of abiotic origin such as PAH, (2) to discern biotic organic compound seen inside cells, and (3) to highlight biotic organic compound surrounded by membrane. In other words, our microscope is capable of identifying what we think to be the most fundamental features that a cell should possess to constitute life.

In characterizing life-related organic compounds, the spatial resolution that a microscope needs to have is about 1 micrometers/pixel. This resolution is much higher than any microscope onboard Mars missions but stems from our knowledge of terrestrial life. The required spatial resolution allows us to image the fundamental feature of life. Even in the case where no detection turns out to be the conclusion of the exploration by our instrument, because of the high-sensitivity, it will set a scientifically meaningful upper-limit for understanding habitable condition in the universe. The upper-limit information will be critical with regard to protecting human being from bacterial infection when setting on Mars is planned.

Refferences: [1] Biemann, K., et al. (1977) *J. Geophys. Res*., 82: 4641-4658 [2] Carr, M.H. (2007) Surface of Mars, Cambridge University Press, 322pp [3] Carr, M.H. and J. W. Head (2003) *J. Geophys. Res.*, 108 (E5), 5042, DOI: 10.1029/2002JE001963 [4] Carr, M. H. and J. W. Head (2010) Earth and Planet. Sci. Lett., 294, 185-203 [5] Head et al., (1999) Science 286, 2134-2137.[6] Jakosky, B. M. and R. J. Phillips (2001) Nature 412, 237-244 [7] Bibring, J. P. et al., (2006) Science 312, 400-404. [8] Acuna, M. (1999) Science 284, 790-793 [9] Leshin et al (2013) Science 341:1238937 [10] Meslin, P. –Y et al (2013) Science 341: 1238670 [11] Williams, R. M. E. et al (2013) Science 324, 1068-1072 [12] McEwen, A. S. et al (2011 Science 333, 740-744 [13] McEwen, A.S. et al (2013) Nature Geoscience 7, 54-58

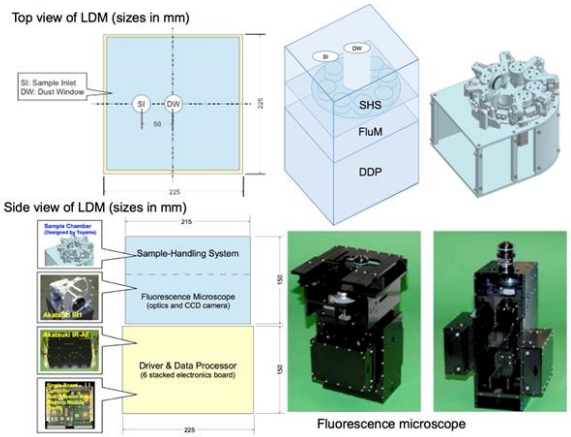

Figure 1: Top, side view of LDM (left) , over vies(center), Sample Chamber (right top), which is placed in Sample Handling System, and Fluorescence Microscope (right bottom). SI: Sample inlet, DW: Dust Window

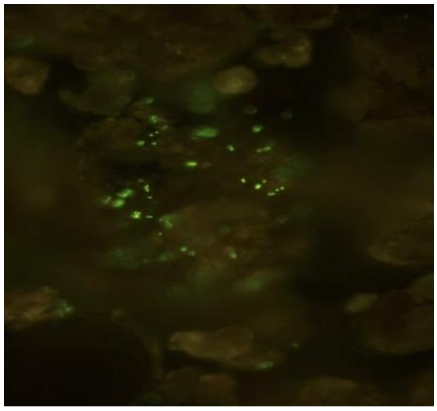

Figure 2 : microbes in the simulated Mars soil (right, green dots) detected by the Life Detection Microscope prototype