FLUORESCENT DYE HANDLING SYSTEM FOR MELOS1 LIFE DETECTION MICROSCOPE

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Introduction: Unlike other missions [1], one of the goals of the MELOS1 mission is to find life directly on Mars. To fulfill this purpose, observation of potentially existing cell images will be performed using a microscope. To distinguish 1 micro-m sized cells from sand particles, fluorescent dyes are planned to be used together with an excellent fluorescent microscope. Use of different types of dyes that can stain nucleic acid, cell membrane, or protein-like molecules will be used. Detection of a wide variety of organic compounds would be able using the same method. To operate unmanned microscopic observation on Mars, one bottleneck would be the handling of dye solution. Concerning the temperature and pressure on Mars, choice of dye solvent and the way of its supply to the collected sample sand should be carefully performed. Here we report the method of dye solution supply onto the sampled sands. Effect of material property (wettability) on solution dropping will also be reported.

BACKGROUND

Microscopic detection of bacterial cells is often performed using fluorescent dyes that can stain biomolecules. Handling of the dye solution through the cold journey to Mars is,therefore, important. Although the temperature on the red planet is usually below 0 C, our life detection microscope (LDM) can share the heat for rover that would keep itself in operation during the night. As the rover is solar powered, LDM is expected to operate during the day time. In this view, selection of the solvent that stays at liquid state at ca. 0 C is needed. Ethyleneglycol and methanol, for example, are widely used material to promote antifreezing property of a water-based solution. As measurement of enzymatic activity is planned in MELOS1 mission, they are not suitable because they might cause denaturation of some proteins. Glycerol, on the other hand, show freezing point reduction effect when mixed with H2O. It is also used to keep bacteria dormant for several years. Freezing point and vapor pressure can be calculated using physical chemistry. In addition, viscoelasticity of the glycerol-water mixture should be focused on, as the dye solution must smoothly mixed with the Mars sand sample.

OBJECTIVE OF THIS STUDY

(1) Estimation of the freezing point and vapor pressure of glycerol solution at Mars environment.
(2) Examination of the effect of (i) hole size (ii) solution level that enable the drop of solution (without pressure difference force).

Mars

Temperature
at the equator in the summer : 20ºC
at the poles : -153ºC
In the mid-latitudes (average) -60 ~ 0ºC

The average air pressure
7.5 millibars = 750 Pa
(can vary by as much as 50%)
[http://quest.nasa.gov/aero/planetary/mars.html]

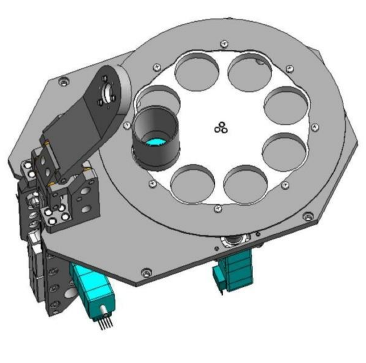

Fig. 1 Sampler

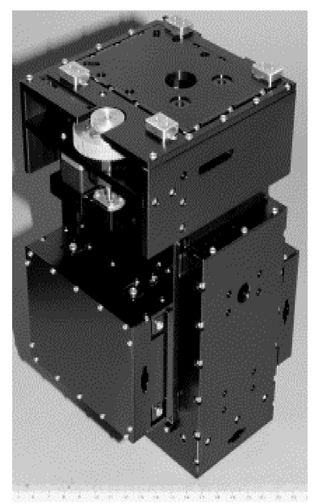

Fig. 2. Microscope housing. Field lens is designed to move vertically to find a focal point. Dichroic mirrors are used to have fluorescent images.

ESTIMATION

All the estimation was performed for 30% (by weight) solution. Fig. 3 shows the relative vapor pressure (relative vapor pressure) with glycerol concentration, based on the reported data.Five data points were smoothly fitted by a curve for the calculation of the 30% glycerol sollution RVP.

EXPERIMENT

Fig. 5 shows the scheme of life searching on Mars. After sampling the Mars sample sands by the use of robot arm of the rover, sample cuvette will be sealed. The cuvette will then separated by the metal foil with the dye solution-capsule that contains Earth atmosphere (100 kPa). A claw will rip the foil and the solution will drop over the sampled sands (Fig. 1). Drop behavior of the solution depends on the foil wettability, surface tention of the solution, thickness of the solution, of the pressure difference between the two spaces. Two different forces, therefore, can be expected to push the dye solution; (1) pressure difference, and (2) gravity (Fig. 6). In this study, we focused solution drop by gravity only.As the behavior of the liquid on surface is often affected by the wettability of the material, we examied the effect of hydrophobicity of the metal surface. Boehmite treatment (Fig. 7) is a simple method to promote hydrophilic characteristic of an aluminum foil. (Fig. 8 は result and discussion) Both treated and untreated aluminum foil were used for the experiment. Solution drop experiment was performed as illustrated in Fig. 9. Al foil at the bottom of the cuvette was penetrated by a drill to have a hole at the center. 30% glycerol solution was added by 100 micro L each time, and the solution level h at the point of solution drop was recorded as "threshold of solution height".

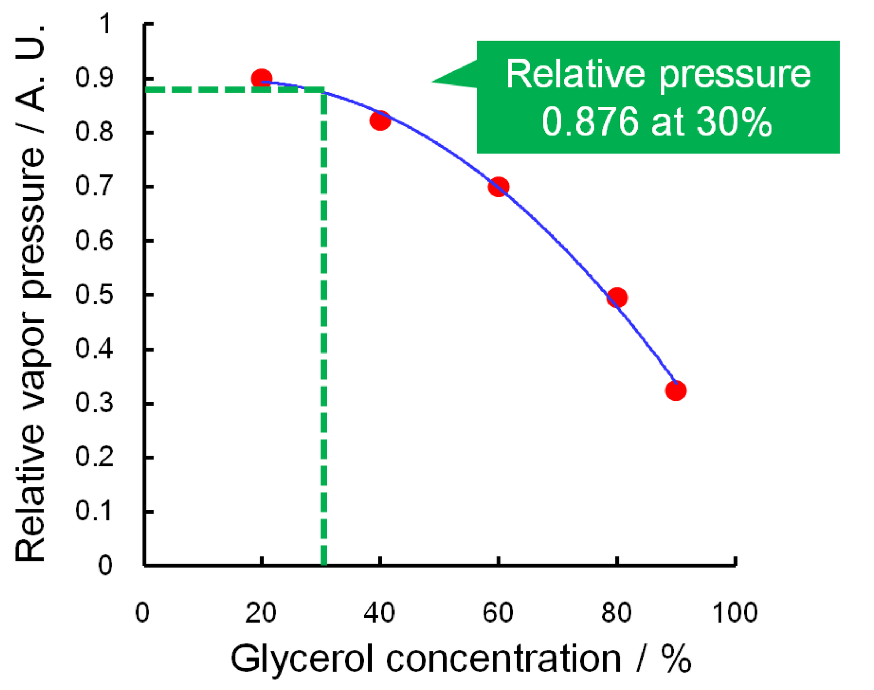

Fig. 3. Relative pressure of glycerol solution. Curve fitting was performed using published data [*]. Approximation function of $y = -0.0001x^2 + 0.0032x + 0.87$ was used, where y = (relative vapor pressure), x = (glycerol concentration in weight per volume).

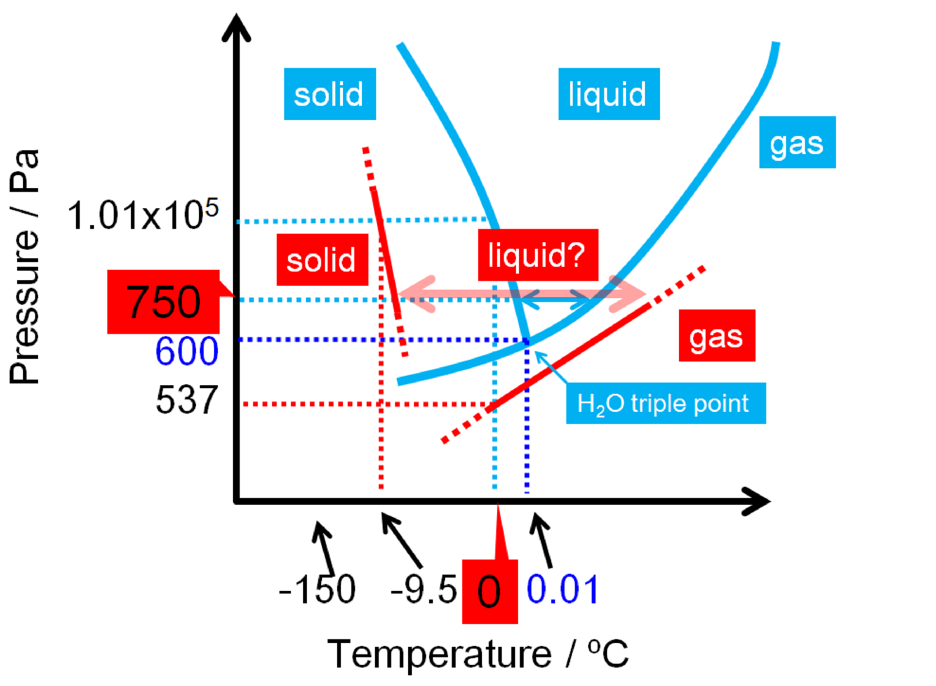

Fig. 4. Schematic phase diagrams of H2O and 30% glycerol solution.Possible phase boundary for the 30% glycerol solution were drawn in red.

30% glycerol solution at 0ºC ...

Freezing point: -9.5ºC [*]
-9.5ºC < 0ºC · not frozen at 0ºC

Vapor pressure:
Dühring's constant for 30% glycerol solution at 0ºC
K=0.876
➔ Calculated vapor pressure of for 30% glycerol solution at 0ºC
P=K x (v.p. of H2O at 0ºC) = 0.876 x 613 Pa = 537 Pa < 750 Pa
not evaporated at 0ºC

...is **liquid** on Mars

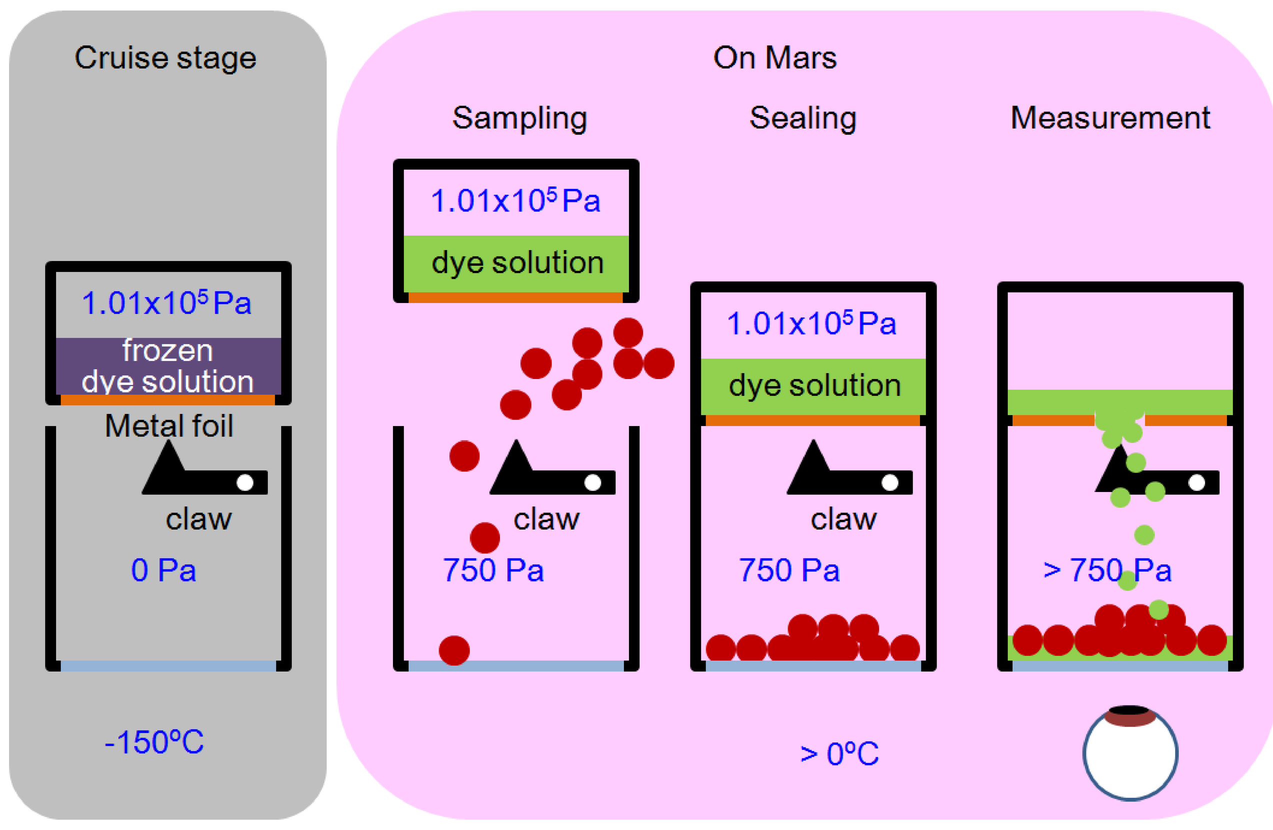

Fig. 5. An example of dye solution handling procesure.Sampling procedure will be followed by sealing and measurement. Dye solution addition (dropping) onto the sample will be performed after sunrise

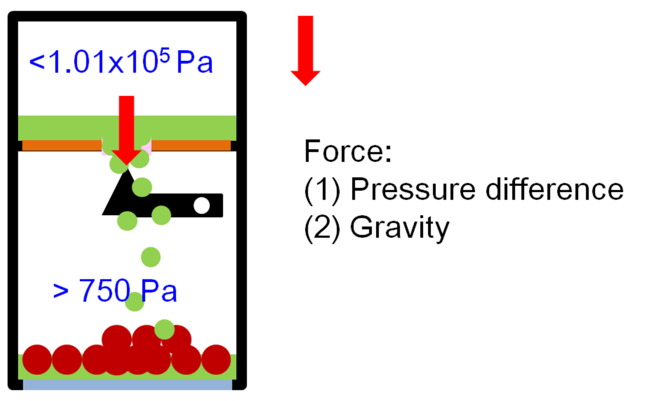

Fig. 6. Available forces to drop dye solution onto the samples. (1) Pressure difference between sample cuvette and dye container. (2) Gravity on Mars (ca. 1/3 of the one on Earth).

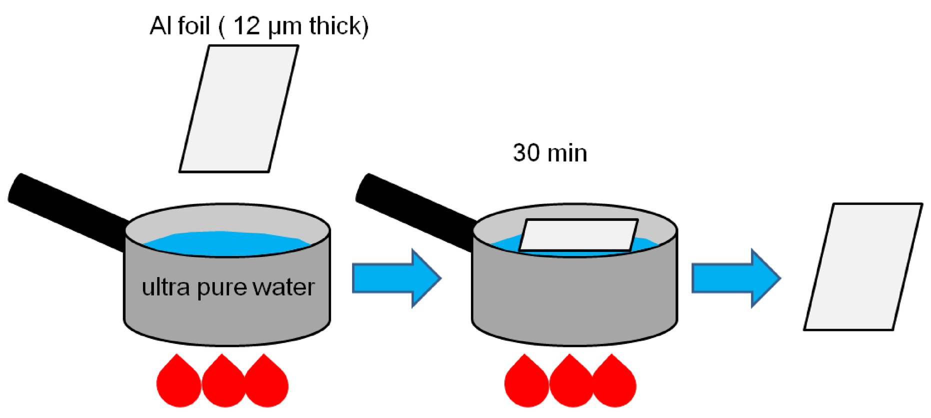

Fig. 7 Boehmite treatment proecess.

Fig. 8. Wettability of Al foil with boehmite-treatment (left) and the one without (right).

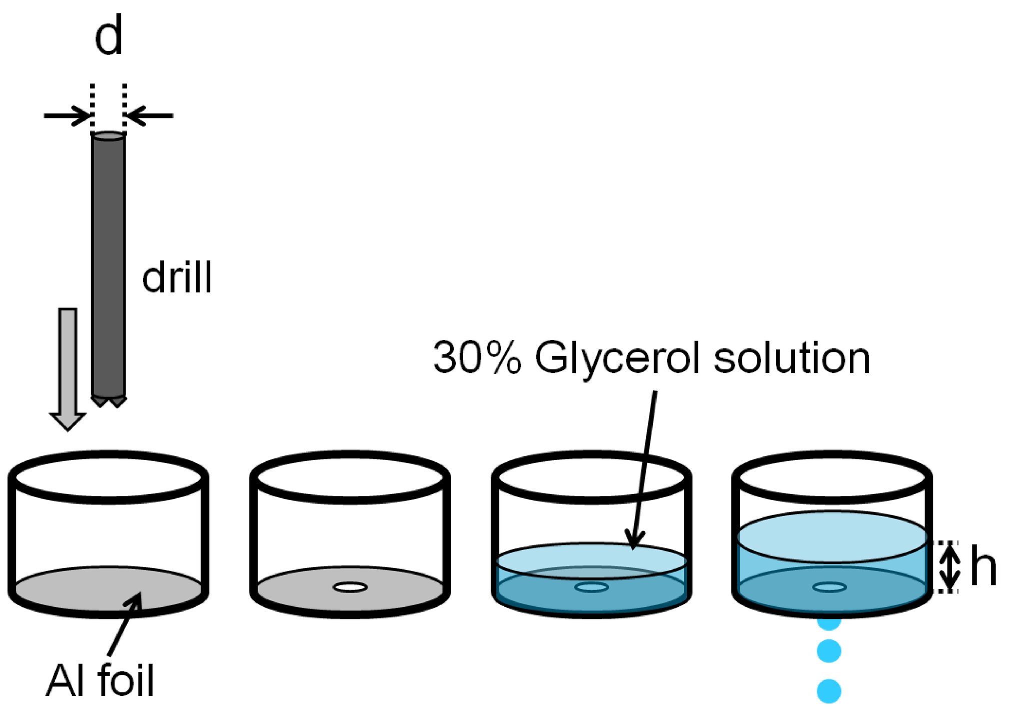

Fig.9. Experimental setup. A hole was made at the center of an Al foil that covered the bottom. 100 micro L of 30% glycerol solution was added at a time. The addition was repeated, and solution level when dropping occured was defined as "threshold of solution height".

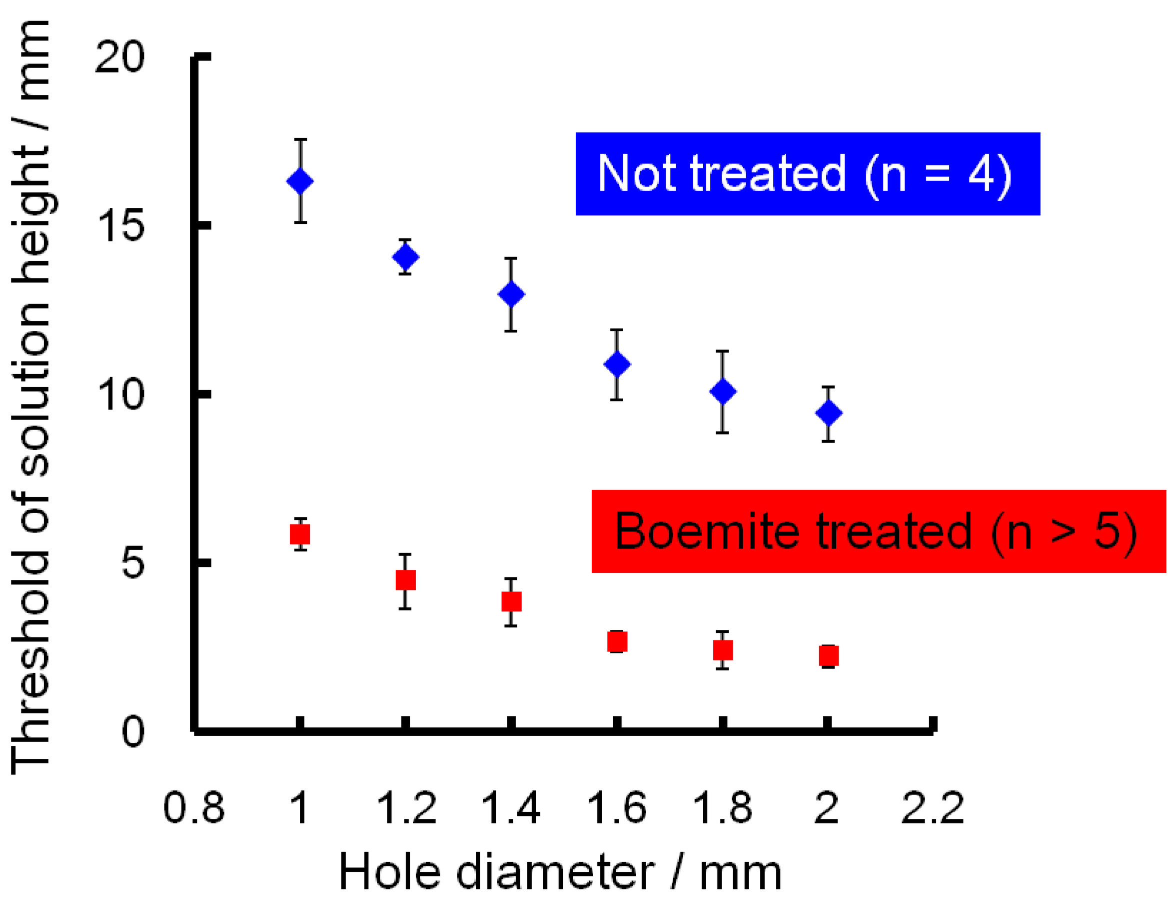

Fig. 10 Effect of metal foil surface treatment on the relationship between the hole size and solution height.

RESULT

Wettability change of Al foil surface after the boehmite treatment was seen in the picture (Fig. 8). After the treatment a water drop on the foil apparently spreaded out wider.In Fig. 10 the relationship between hole diameter and the threshold of solution height was shown. The threshold decreased with the hole diameter in both cases. Considereing the length of error bars (+/- standard deviation), the dropping process was reproducible. With all the hole diameter tried, the treated case showed smaller threshold.

DISCUSSION

In our preliminary study, behavior of the 30% glycerol solution was almost the same with water (data not shown). As is clear from the results in Fig. 10, boehmite treatment worked effectively from the solution dropping viewpoint.Generally the detouchment of water drop is easier from the hydrophobic than from hydrophilic surface, but our results showed that later surface was favorable for the dropping process. The solution was indicated to require surface attachment environment to penetrate through the hole, because liquid-solid interface area tends to be smaller when the surface is more hydrophobic. Larger work was therefore necessary to increase the interface area compared to the case with a hydrophilic surface.

CONCLUSION

In our study 30% glycerol solution was indicated to be a candidate for the dye in LDM. Hydrophilic treatment of the metal foil was thought to be favorable for the dye-dropping procedure.

References: [1] Atreya S. K. 2007. Planet. Space Sci. 55: 358-369. [2] Hart R. K. 1953. Trans. Faraday Soc. 50: 269-273. [3] Sefiane K. 2004. J. Colloid Interface Sci.15: 411-419.