

**TESTING METHODS FOR DETECTION OF UNFAMILIAR LIFE IN MARTIAN REGOLITH** Ari H.D. Koeppel<sup>1</sup>, David Trilling, George Koch, Egbert Schwartz, Christopher Edwards, Northern Arizona University, Flagstaff, AZ 86011, <sup>1</sup>(akoeppel@nau.edu);

**Introduction:**

Mission-related efforts to detect extant life on Mars' surface, such as the Viking lander experiments [1], have thus far focused on organic chemical tracers and gaseous byproducts of active biotic processes. Yet, despite the multiple experiments conducted on Mars' surface, consensus on the evidence for extant life remains missing [2]. Scientists and philosophers have speculated on the possibility that Martian life takes a form that is chemically unfamiliar to us and to the design of those early experiments [e.g. 3,4]. As such, a method of detecting extant life that does not require organic chemistries may be a useful complement to the more conventional approaches on future missions to Mars' surface.

In this exploratory work, we investigate and compare the viability of two easily coopted lander payloads for detecting biotic activity in Martian soils which do not rely on C-O-H chemistries. We present and discuss results from initial sensitivity tests of cellular metabolism using the non-toxic redox tracer dye Alamar Blue (resazurin, ThermoFisher) [5,6] and infrared thermography [7] in an inoculated Mars regolith simulant [8].

**Methods:**

*Alamar Blue (resazurin)*

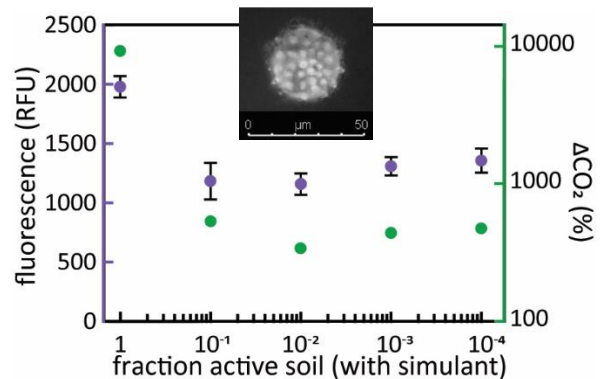
Alamar Blue experiments were conducted in sealed jars filled with mixtures of biologically active and Mars simulant soils in a factor-of-ten dilution series. Upon reduction, non-fluorescent resazurin becomes highly fluorescent resorufin. After mixing samples with Alamar Blue, fluorescence was measured using a plate reader and qualitatively confirmed using a confocal fluorescence microscope. As points of comparison, we also measured CO<sub>2</sub> at the beginning and end of the experiment. In both experiments we controlled for grain-size, water content and basaltic provenance in order to limit sample variability.

*Infrared Thermography (IRT)*

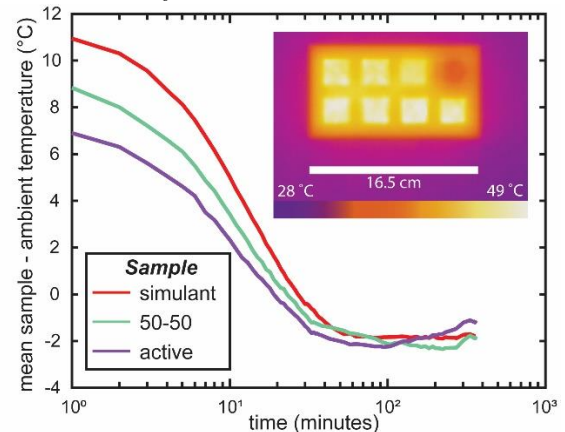
Eight mixtures of Mars simulant and active soil were placed on a partitioned sampling tray. After mixing 1.5 mL of distilled water into each sample and heating the samples in a 45 °C oven for 8 hours, the tray was placed in a 30 °C dark chamber with a downfacing infrared (7.5-13.5 μm) imager overhead for 6 hours. Pixel values converted to temperature provide a time series.

**Results and Discussion:**

Reconnaissance testing using Alamar Blue solution suggests that biotically-triggered fluorescence can be detected (Fig. 1) with similar sensitivity as traditional gas exchange monitoring, although future testing with less dilute mixtures will improve interpretations.



**Fig. 1.** Alamar Blue-activated fluorescence (excitation, Texas Red filter at BP 560/40) and  $\Delta\text{CO}_2$  in soil samples diluted with Mars simulant. Uncertainty on  $\Delta\text{CO}_2$  measurements is smaller than the marker sizes.



**Fig. 2.** IRT-derived temperature evolution of 3 of 8 samples. Maximum uncertainty is  $\pm 0.5$  °C.

IRT experiment (Fig. 2) show that samples with larger fractions of active soil show lower temperatures after heating and higher temperatures at ambient ( $\sim 30$  °C) thermal equilibrium. If the temperature variation is a result of biotic activity, initially lower temperatures may result from cellular fluid retention, while the eventual warming may result from metabolic reactions [9].

This and prior works indicate that both Alamar Blue and IRT have the potential to be useful, non-destructive tools for the detection of extant life in Martian regolith. Further investigations will provide better constraints on the sensitivity of these tools to the variety of possible forms of life that may exist on Mars.

**References:**

- [1] Klein, H.P. (1978) *Icarus* 34 (666–674) [2] Benner, S.A. *et al.* (2000) *PNAS* 97 (2425–2430) [3] Cleland, C.E. (2012) *Synthese* 185 (125–144) [4] McKay, C.P. (2004) *PLoS Biol.* 2 [5] O'Brien, J. *et al.* (2000) *Eur. J. Biochem.* 267 (5421–5426) [6] Rampersad, S.N. (2012) *Sensors (Switzerland)* 12 (12347–12360) [7] Kluge, B. *et al.* (2013) *Soil Biol. Biochem.* 57 (383–389) [8] Cannon, K.M. *et al.* (2019) *Icarus* 317 (470–478) [9] Russell, J.B. & Cook, G.M. (1995) *Microbiol. Rev.* 59 (1–15)