

TESTING METHODS FOR DETECTION OF UNFAMILIAR LIFE IN MARTIAN REGOLITH



Ari H.D. Koepfel¹ (akoepfel@nau.edu), David Trilling¹, George Koch², Egbert Schwartz², Christopher Edwards¹

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Background

Mission-related efforts to detect extant life on Mars' surface have thus far centered on geochemical tracers for active biotic processes. The Viking landers carried a suite of tools for measuring organic molecules and gaseous byproducts of metabolism [1]. Yet despite the multiple experiments conducted on Mars' surface, consensus on the evidence for extant life remains missing [2].

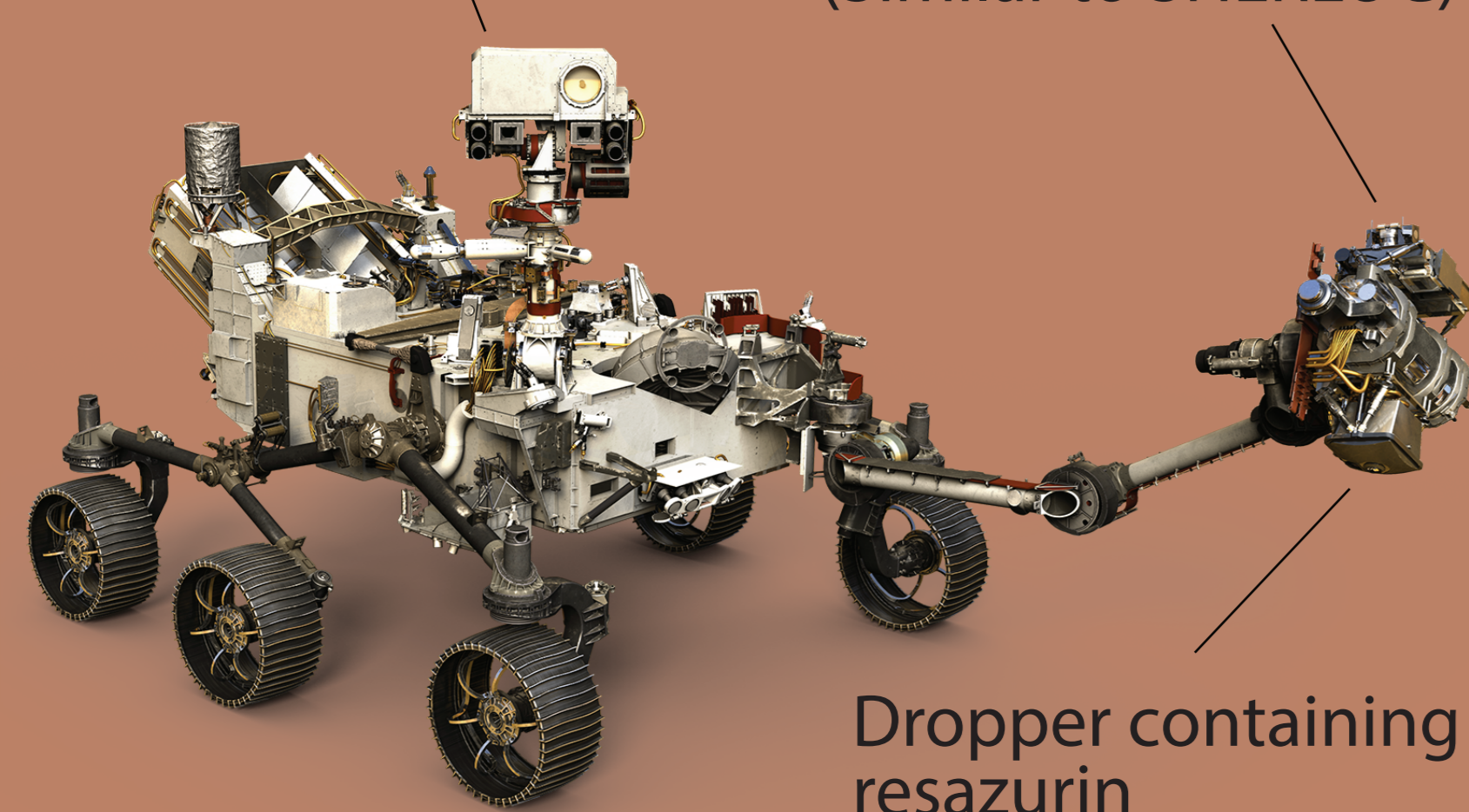
Scientists and philosophers have speculated on the possibility that Martian life takes a form that is chemically unfamiliar to us and to the design of those early experiments [e.g. 3, 4]. As such, a method of detecting extant life that does not focus on known organic chemistries may be a useful complement to more conventional approaches.

These efforts may be guided by our understanding of possible metabolic processes [5-7]. As such, we seek tools that can detect evidence of fundamental features of metabolism: thermodynamic disequilibrium and electron transfer.

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 known Earth chemistries. We present and discuss results from initial sensitivity tests of cellular metabolism using the non-toxic redox tracer dye Alamar Blue (resazurin, ThermoFisher) [8,9] and infrared thermography [10] in inoculated Mars analog regolith [11].

Infrared imager capable of capturing >1 image/10 minutes (1-14 μm). Part of Mastcam

Fluorescence-inducing laser and spectrometer at BP 560/40 (Similar to SHERLOC)



Dropper containing resazurin

Figure 1. Mars 2020 rover labelled with proposed and existing instrument concepts (adapted from NASA).

We demonstrate the capabilities of two potential rover payloads to recognize biotic activity in Martian soils. These approaches are agnostic to known organic chemistries.

Results

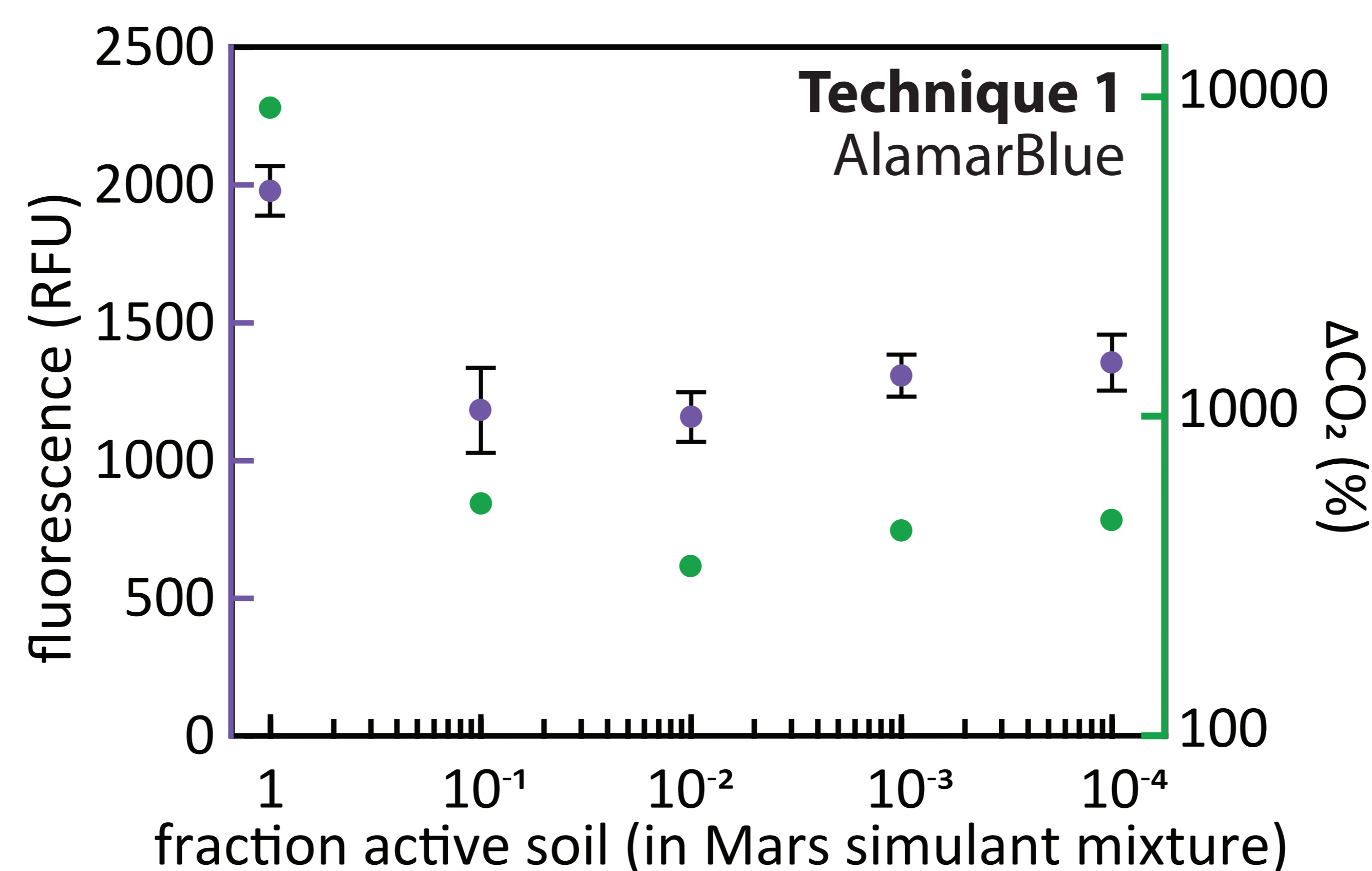


Figure 2. AlamarBlue-activated fluorescence in soil samples. Fluorescence (relative fluorescence units) in purple and ΔCO_2 in green after incubation for organic soil samples diluted with Mars simulant. Results show similar levels of sensitivity to metabolic activity between Alamar Blue and the more conventional CO_2 production measurement methods. Uncertainty on ΔCO_2 measurements is smaller than the marker sizes.

Technique 2: Infrared Thermography

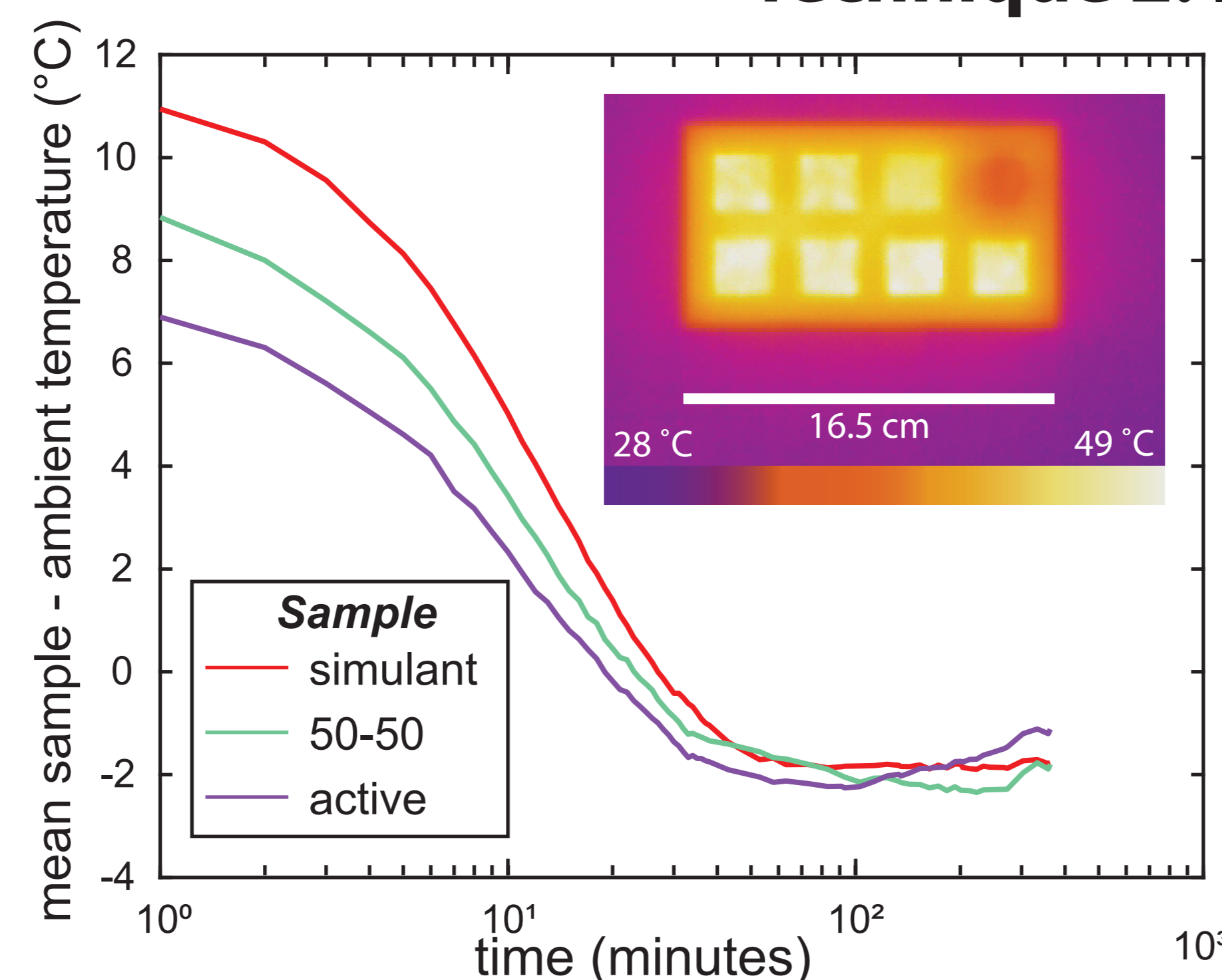


Figure 3. Infrared thermography (IRT)-derived temperature evolution of 3 of 8 samples after transfer from 45 °C to 30 °C ambient. Maximum uncertainty is ± 0.5 °C. Inset is an example calibrated temperature image of sample tray.

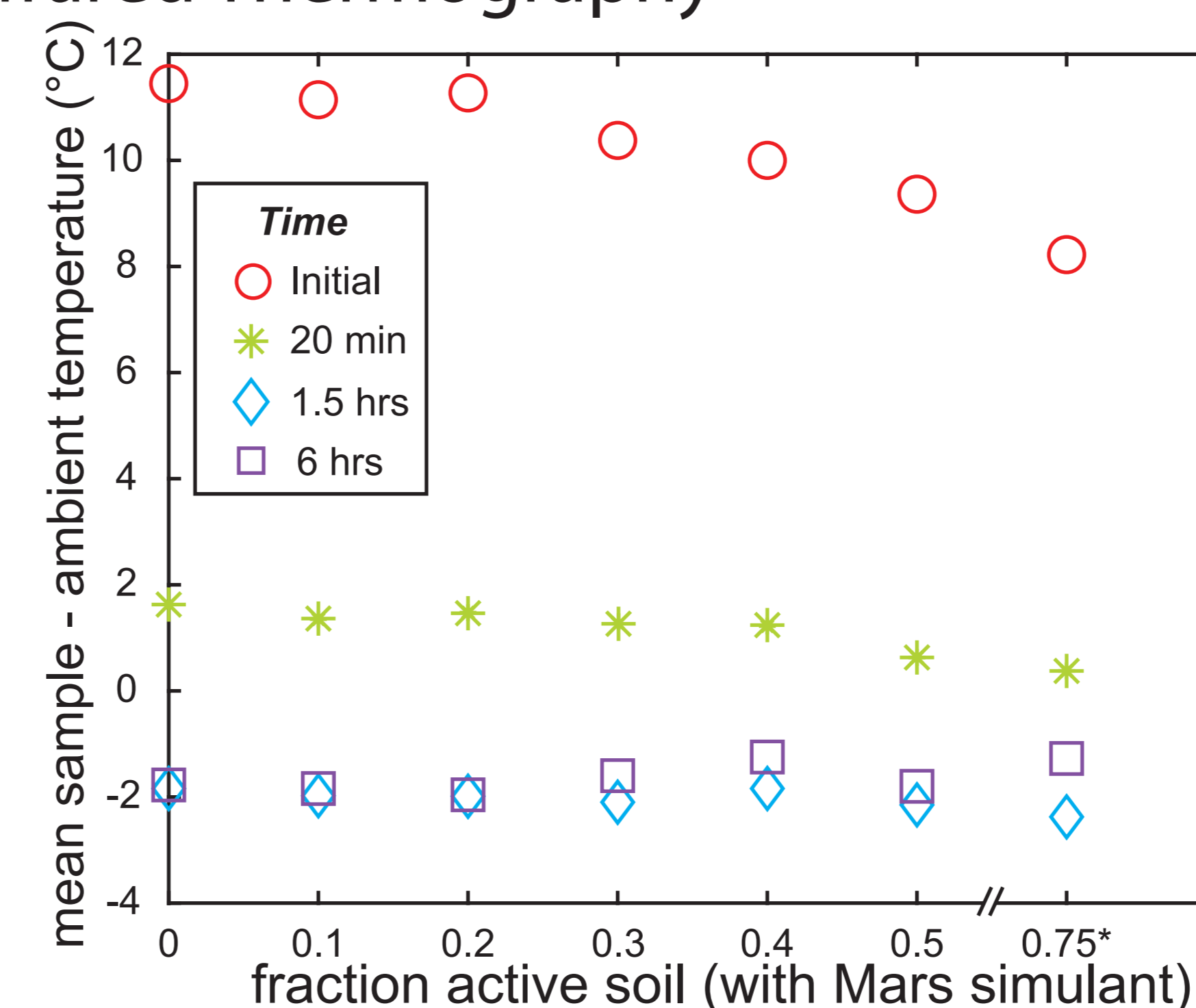


Figure 4. Time snapshots of temperature for 8 sample mixtures. Far left is 100% Mars simulant, far right is 100% Red Mountain soil. *sample is 75% red mountain, 25% garden soil mixture.

Techniques

Alamar Blue

Experiments were conducted using 200 g soil samples placed in sealed 1 L glass jars. Jars were filled with mixtures of active natural and Mars simulant soils in a factor-of-ten dilution series (jars contained 100%, 10%, 1%, 0.01%, and 0.001% active soil). Active soil refers to organic soil collected from the Red Mountain basaltic volcanic edifice northwest of Flagstaff, AZ and sieved to 2 mm grain size. Mars simulant refers the Exolith Lab Mars Global Simulant-1 [11].

After a 48 hr incubation period, we opened each jar and removed 0.5 g subsamples of soil and mixed each with 1 mL of Alamar Blue resazurin solution. Upon reduction, non-fluorescent resazurin becomes highly fluorescent resorufin. After 4 hours of incubation, we pipetted solution from each sample into partitioned plate cups. Fluorescence of each sample was then measured using a BioTek Synergy HT Plate Reader (via excitation, Texas Red filter at BP 560/40). We also imaged incubated samples using a Leica TCS SPE II confocal fluorescence microscope to qualitatively confirm that fluorescence was rcorrelated with cellular activity.

As points of comparison to Alamar Blue testing, we also measured CO_2 concentrations (LiCor 6262 gas analyzer) in the jar airspace at the beginning and end of the experiment.

Infrared Thermography (IRT)

Samples of Mars simulant and dried Red Mountain soil were placed on a partitioned 16.5 cm x 9 cm sampling tray in seven 7.5 g mixtures (100%, 90%, 80%, 70%, 60%, 50%, and 0% Mars simulant). An eighth slot was filled with 75% Red Mountain soil and 25% high-organic-content garden soil. 1.5 mL of distilled water was mixed into to each sample and the tray was placed in moisture-sealed packaging in a 45 °C oven for 8 hours.

After removing the samples from the oven and packaging, the tray was placed in a 30 °C dark chamber 80 cm beneath a downfacing FLIR Duo pro infrared (7.5-13.5 μm) imager. The camera captured one stationary image of the sample tray every minute for 6 hours. By isolating the pixels encompassing each sample (approximately 500 pixels each), and averaging their converted temperature values, a temperature time series was produced for each sample.

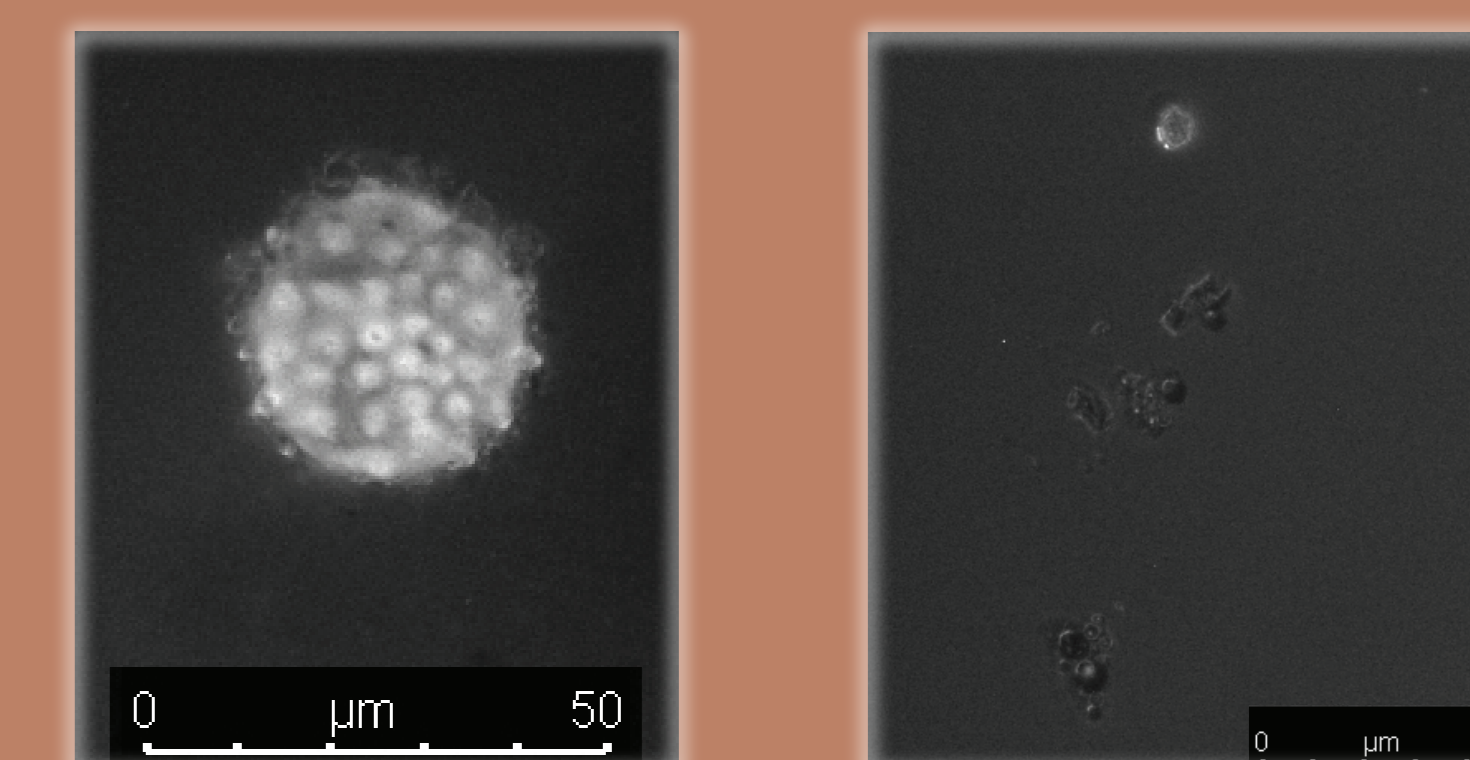
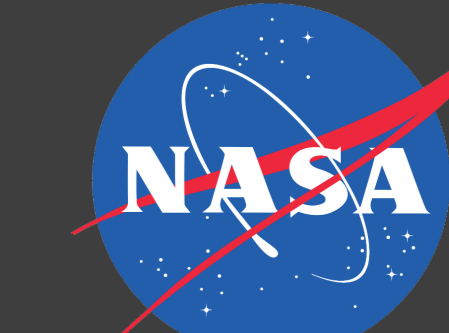


Figure 5. Alamar Blue-activated cells under a fluorescence microscope appear bright while abiotic material appears darkly silhouetted.



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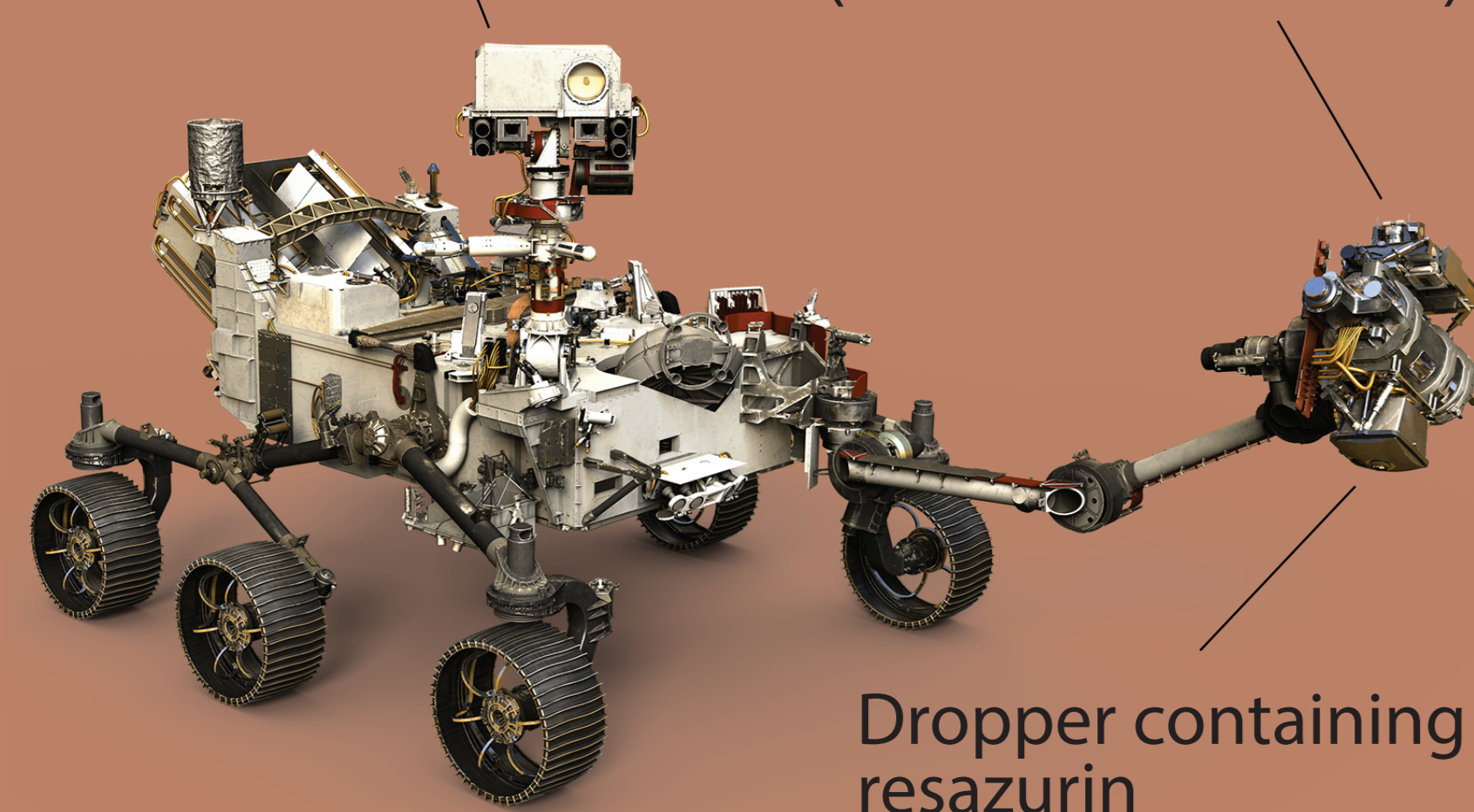


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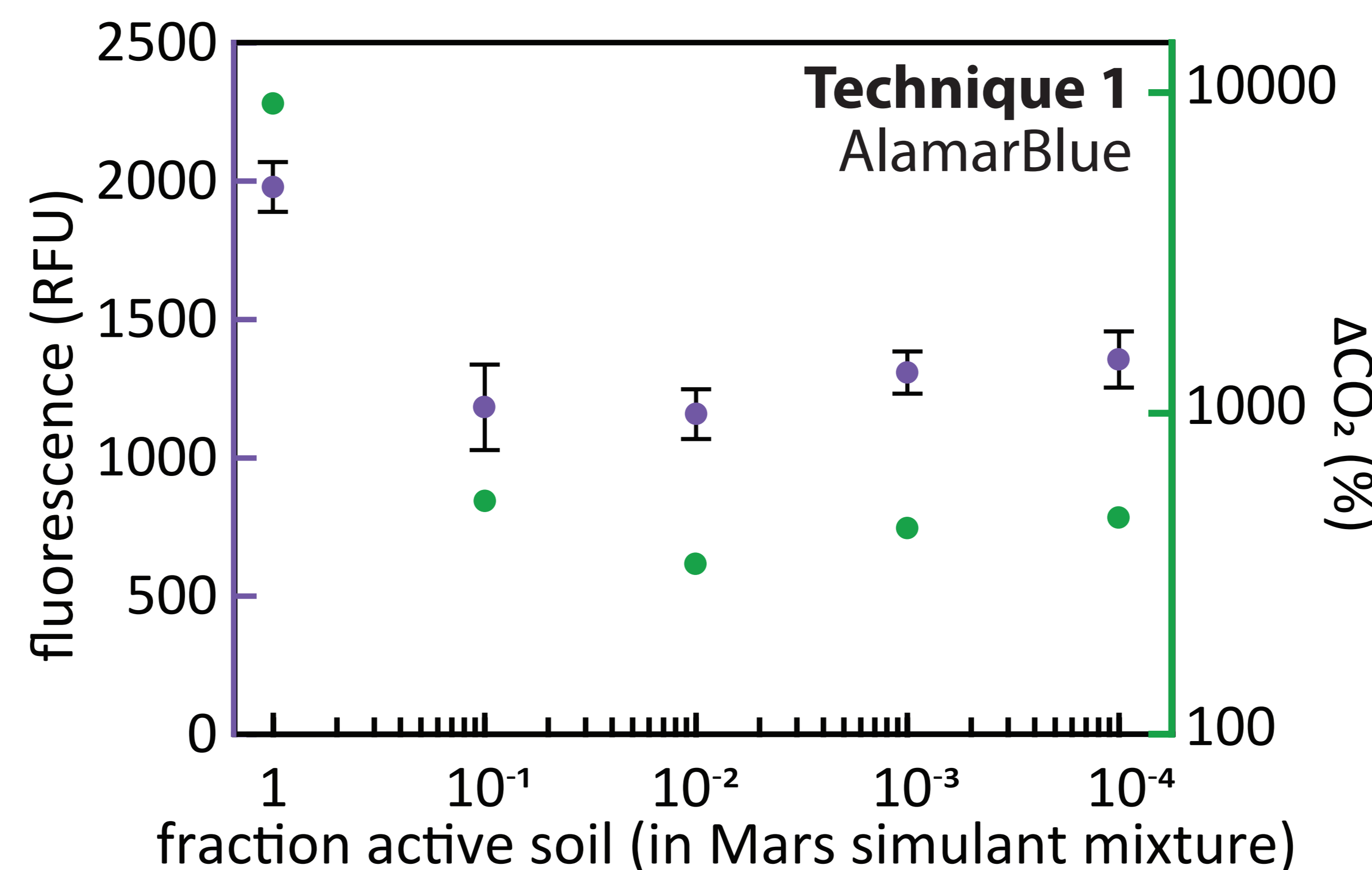


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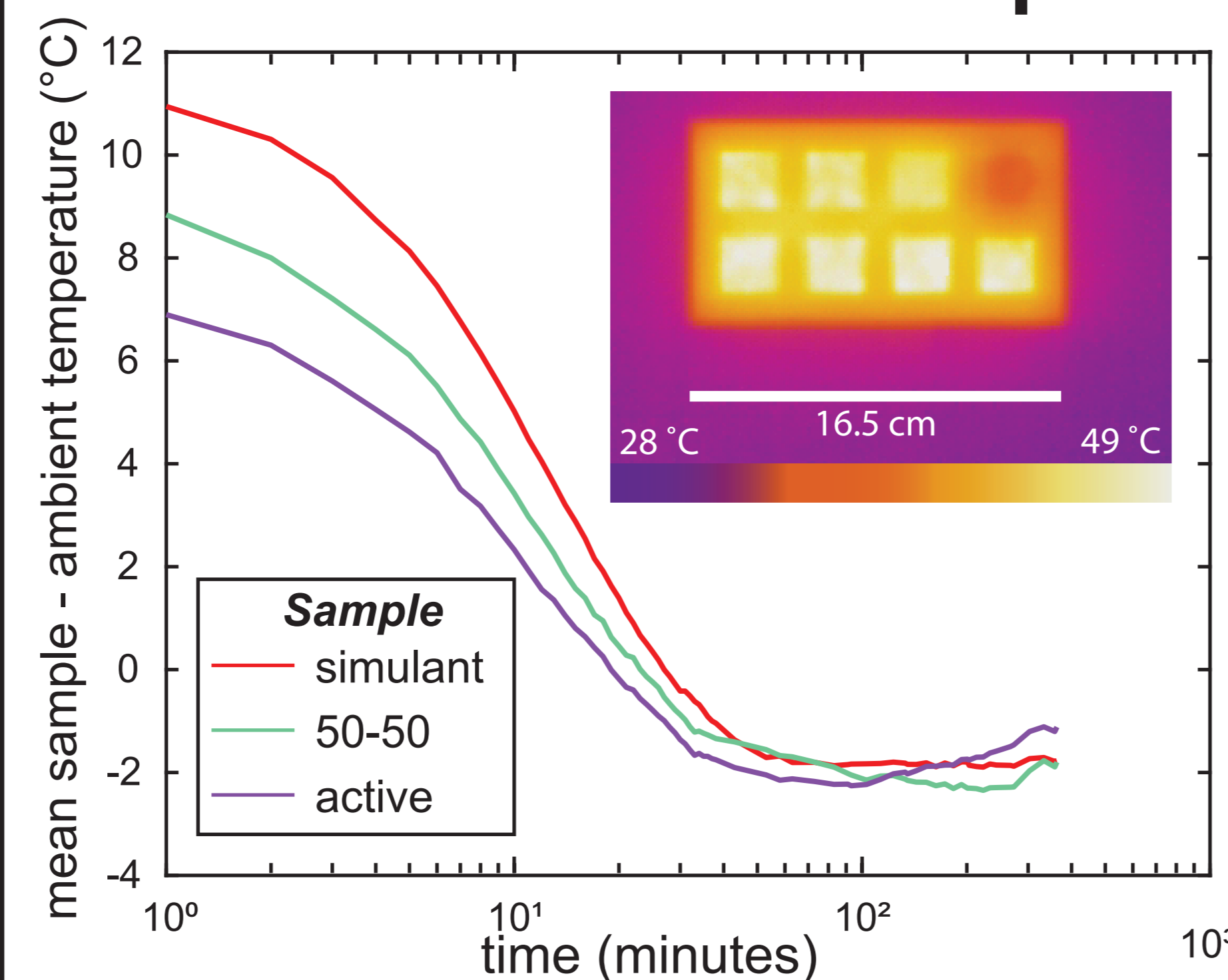


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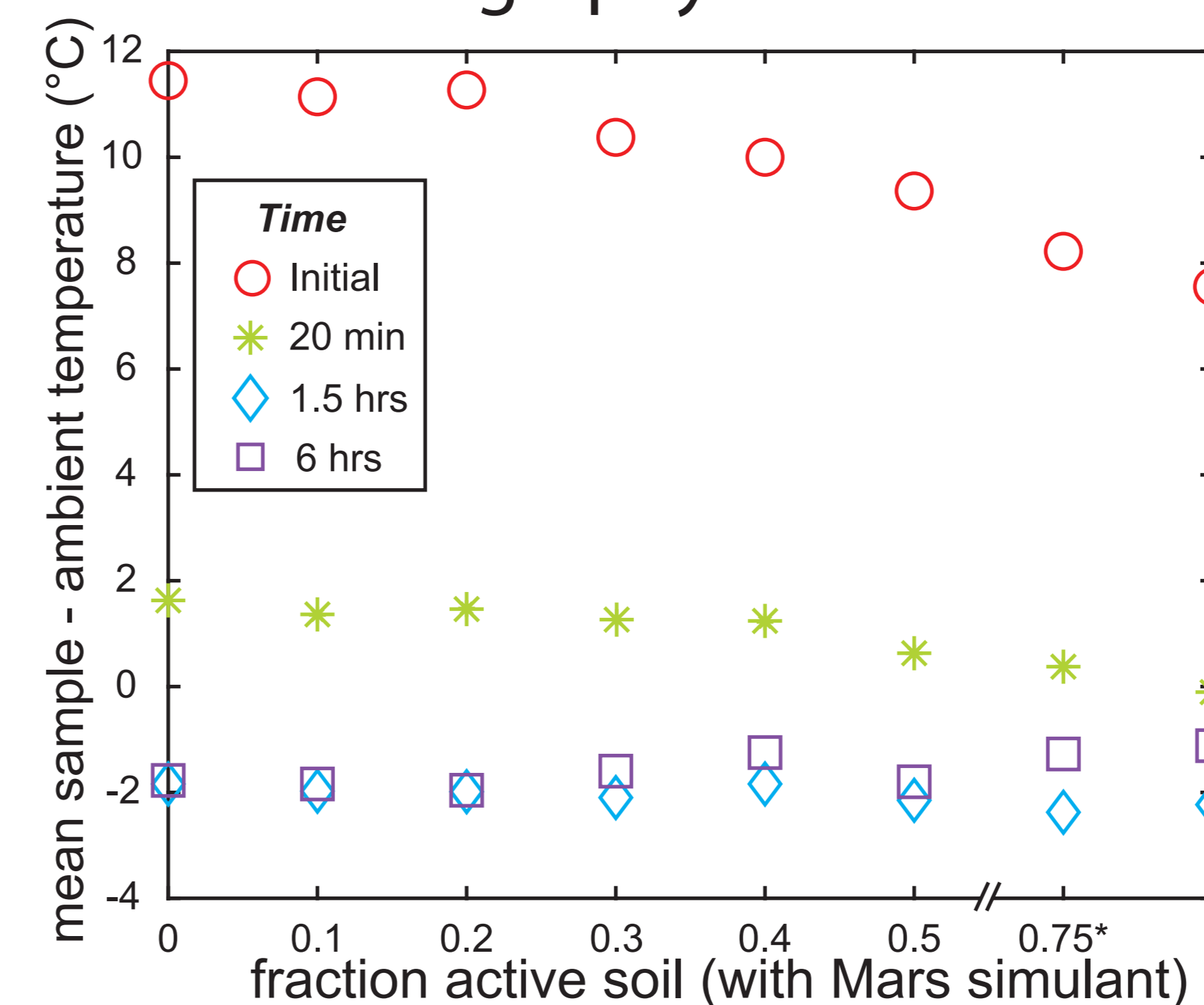


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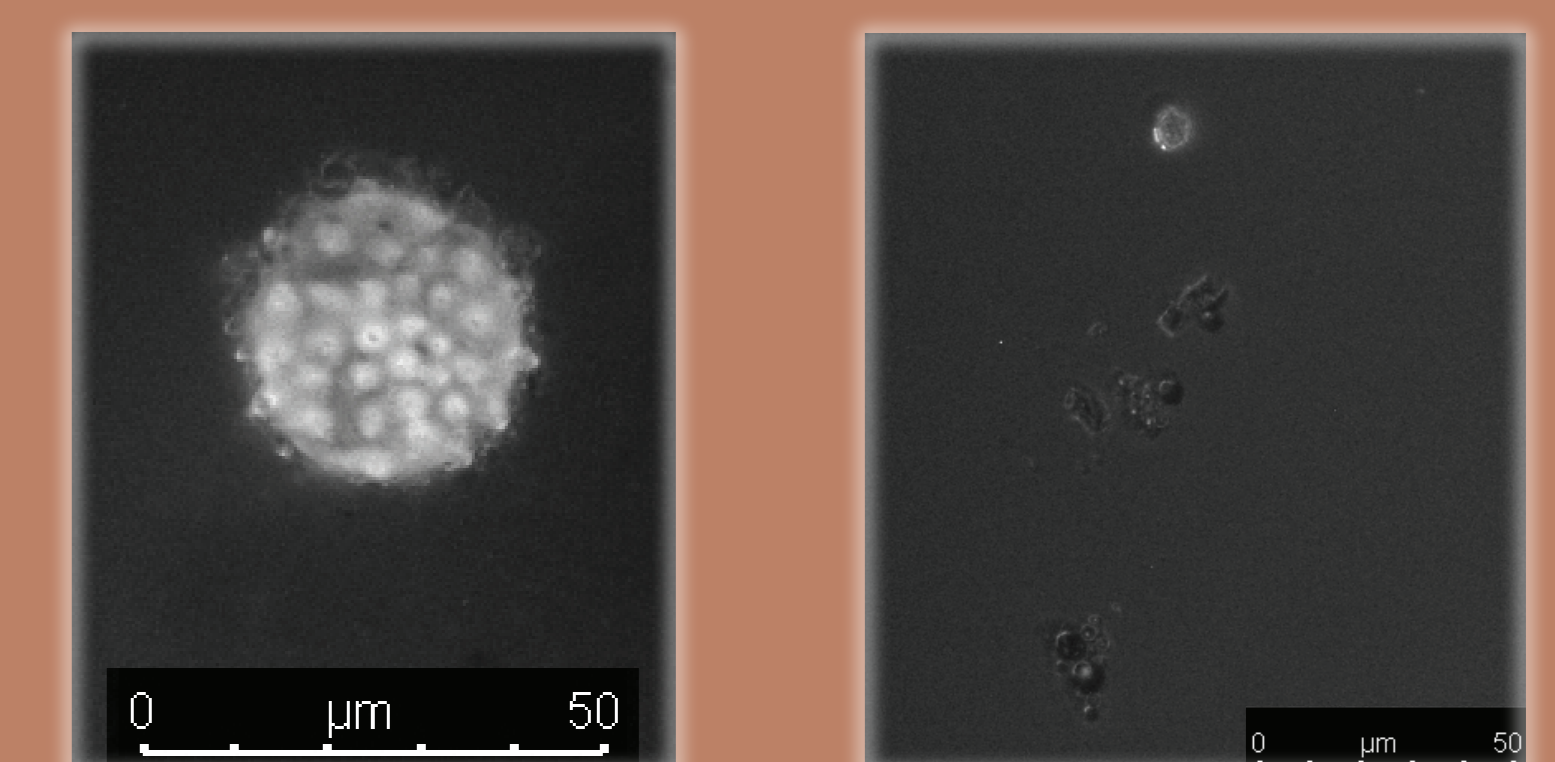


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