A HOLOGRAPHIC MICROSOPE FOR MOTILITY AND TAXIS DETECTION. M. Bedrossian[1], S. Rider[1], J. L. Nadeau[1] and C. A. Lindensmith[2], [1] Graduate Aerospace Laboratories, California Institute of Technology, 1200 E. California Blvd., Pasadena, CA 91125, mbedross@caltech.edu, [2] Jet Propulsion Laboratory, California Institute of Technology, 4800 Oak Grove Dr., Pasadena, CA 91009, lindensm@jpl.nasa.gov.

Introduction:
Extraterrestrial life in our Solar System, if present, is almost certain to be microbial. While we cannot predict extraterrestrial biochemistry, we can be certain that the biochemistry must be isolated from the environment in some way—that is, it will consist of cells(*1, 2*). Because we cannot know in advance what the structural molecules of extraterrestrial organisms might be, micrometer scale imaging provides a crucial complement to techniques such as mass spectrometry. This imaging must be performed at the micrometer or submicrometer scale because prokaryotic life is limited by nutrient diffusion into the cell, restricting most bacteria and archaea to sizes 0.4-2 µm.(*3*). Similar cellular scales would be expected on icy moons, where the liquid water environments have similar composition and temperatures to Earth.

Methods and technologies for unambiguous detection of living, (or recently alive but killed or dormant) prokaryotes are lacking, and thus critically needed for life-detection missions(*4, 5*). As exploration of our Solar System widens to include more likely habitats for extant microbial life than the ancient, dry terrain of Mars, direct imaging of microorganisms becomes more and more attractive as a target, one that is beginning to attract interest in the astrobiology community. Imaging can answer fundamental questions about life that chemical techniques cannot. Direct observation of cells directly addresses key aspects of the nature of life: particularly whether extraterrestrial life is cellular in the way we currently understand it on Earth, and what structure cells assume. Put simply, imaging answers the question “What does extraterrestrial life look like?”

Imaging of individual prokaryotic cells can be ambiguous, even with extremely high-resolution techniques such as electron microscopy. Rather than maximize spatial resolution, we believe that imaging prokaryotes should maximize throughput and provide temporal information, allowing for observation of context and of complex behaviors such as motility and taxis. Our primary hypothesis is that microbial motility is an inherent feature of natural aquatic habitats, even extreme (subzero temperature) ones, with even the most extreme environments expected to show cell counts of 10^4-10^5 cells/mL. Not all inhabitants may rely upon movement to complete their life histories, but some fraction of the community will have evolved the ability to achieve directed motion via swimming or gliding. If not swimming at the time of *in situ* imaging, a controlled shift in some aspect of the environmental conditions can induce or stimulate motility in enough organisms to enable detection.

Methods and Results : we have built and field-tested a digital holographic microscope (DHM) to TRL-5, a unique instrument that shows greater spatial resolution than all previous designs, allowing for imaging of microorganisms down to 800 nm with limits of detection ~10^3 cells/mL, ~100-fold more sensitive that ordinary light microscopy (**Fig. 1 A**). In order to determine how best to stimulate motility, we have performed laboratory experiments on cultured strains and environmental samples and quantified responses to temperature and to gradients of simple sugars and amino acids. We have also designed sample chambers for a flight instrument where multiple stimuli may be applied to a given sample (**Fig. 1 B**). Along with typical stimuli such as amino acids, we are also examining the possibility of using non-Earth-centric stimuli such as terminal electron acceptors (as nanoparticles or electrodes), which have been shown to stimulate activity in anaerobic Earth microorganisms and which represent a general principle that is expected to apply to any organism. Here we present the design of the instrument and sample chambers as well as some taxis results from a variety of analogue environments.

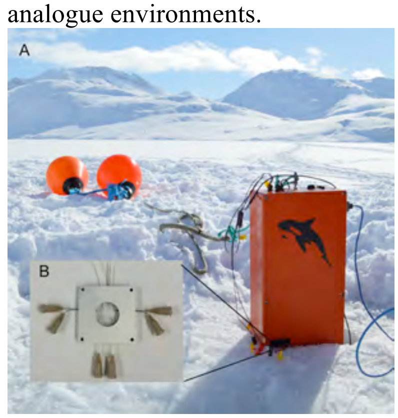

Figure 1. Field-deployed holographic microscope (A) with sample chamber to introduce stimuli (B).

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