ExCALiBR: An Instrument for Uncovering the Origin of the Moon's Organics. M.B.Wilhelm[1] A.J. Ricco[1,2] M. Chin[1,3] J.L. Eigenbrode[4] L. Jahnke[1] P.M Furlong[1,5] D.K. Buckner[1,6] T. Chinn[1] K. Sridhar[1] T. McClure[1,2] T. Boone[1,3] L. Radosevich[1,3] A. Rademacher[1,3] T. Hoac[1,3] M. Anderson[1,7] S. Getty[4] A. Southard[4,7] R. Williams[4] X. Li[4,8] T. Smith[1] O. Podlaha[9] and J. van Winden[10], [1]NASA Ames Research Center, Moffett Field, CA 94034; marybeth.wilhelm@nasa.gov, [2]Stanford University, [3]Millenium Engineering Inc., [4]NASA Goddard Space Flight Center, [5] KBR Inc., [6]Blue Marble Space Institute for Science, [7]Universities Space Research Association, [8]Center for Research and Exploration in Space Science, [9]Shell Global Solutions International.

Introduction: Lipids are organic molecules of great astrobiological interest for future NASA missions to the Moon, Mars and Icy Worlds. Lipids are essential for life as we know it, and likely also required for putative extraterrestrial organisms. These robust organics can be synthesized abiotically, and survive for long periods of geological time. Laboratory characterization techniques are well established but are laborious, operator dependent, and require large volumes of consumables, precluding *in situ* analysis. We are developing an autonomous, miniaturized fluidic system, integrating lab techniques for lipid analysis while minimizing reagent volumes and concentrating organics for analysis, thereby increasing signal-to-noise ratios by orders of magnitude (**Fig. 1**). This system, the Extractor for Chemical Analysis of Lipid Biomarkers in Regolith (ExCALiBR), will enable future organic surveys by extracting and concentrating lipids from approximately 50 grams of regolith using a fluidic and microfluidic sample processor made of materials compatible with non-aqueous solvents required for extraction. ExCALiBR can be deployed on a lander or used on a crewed mission.

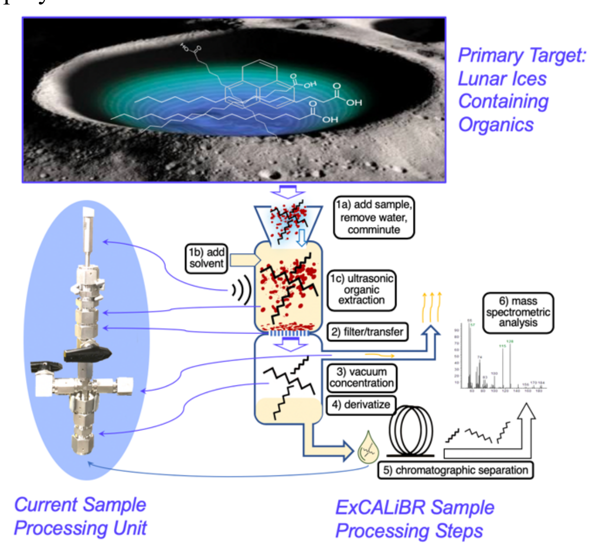

Figure 1: Overview of ExCALiBR primary target, TRL 2 benchtop prototype, and sequence of processing steps that will be integrated and automated to achieve a TRL 5 fluidic instrument.

Science Background & Objective: Lipids are utilized by all life on Earth, primarily to build cell-encompassing membranes that protect cells from water[1]. Lipids are also synthesized via abiotic processes and are detected throughout the solar system, particularly in meteorites and other carbonaceous matter that could have provided the seed material for life to begin on Earth[2-5]. Lipids survive for billions of years in the geologic record on Earth, orders of magnitude longer than any other biomolecule[6]. They also contain origin-diagnostic molecular features (*i.e., number of carbon atoms, chain length, branch points*) that indicate whether they were formed via biotic or abiotic processes. Our science objective is to detect and analyze lipid biomarkers by extracting them from regolith and identifying their origin-diagnostic features.

Lunar Surface Science Motivation: While the terrestrial record of life's origin has been largely erased by geologic processes[7], a repository of the same original organics is believed to exist in topographically low regions of the moon[8]. Recent studies suggest that rapid burial by ejecta material during the late heavy bombardment and subsequent lava flows from lunar volcanism could have preserved them since original deposition/synthesis, as they were protected from radiation[9-11]. Although organics were not identified in samples returned from the Apollo missions[12,13], LCROSS found evidence of organic building blocks in water in the lunar ejecta plume[14]. Given the linked formation of the moon and Earth 4.5 billion years ago[15], characterizing lunar lipids will improve our understanding of organics potentially available on early pre-biotic Earth. Such material could provide an "abiotic baseline" for future life detection missions to Mars and Icy Moons. Additionally, it has been posited that Earth-impacted early biological material could have reached the moon[9,16-20].

Instrument Description and Development Approach: Proven "gold standard" laboratory techniques for lipid biomarker extraction are laborious and require consumables. A critical gap will be bridged by developing a system that replicates analytical lab procedures autonomously on a flight-instrument scale with fidelity to original lab techniques. Automated fluidic devices combine controlled handling of liquids with sequential operations and parallelization of replicate processes. By designing such systems to closely interface with both sample-delivery and analytical measurement systems, laboratory analyses can be automated. NASA Ames has a proven track record developing fluidic systems for astrobiology and fundamental biology applications and operating them in outer space[21-27]. Limit of detection (LoD) requirements dictate parameters for concentration and loss of organic material based on sample mass, derived from terrestrial analog sample extraction protocols.

ExCALiBR will extract lipids from a ground rock/ice sample into organic solvent and sequentially prepare the analyte for analysis by downstream flight instruments. We are currently developing a breadboard-level non-aqueous fluidic system capable of extracting lipid biomarkers from planetary samples, performing filtration of the resulting aliquot, and chemically preparing targets for downstream analysis. Processing modules/sequence relationships are illustrated in **Fig. 2**. We will mature our current benchtop model of ExCALiBR (**Fig 1.**) from TRL 2 to 5 over the next two years. The breadboard will be tested with previously-collected planetary analog samples of varying age and minerology.

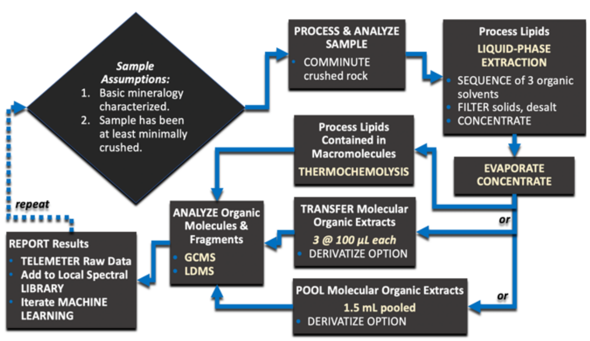

Figure 2. ExCALiBR sample processing sequence and relationships.

Instrument performance will be verified by comparison to "gold standard" laboratory techniques using GSFC flight-analog or flight-prototype analytical instruments. Leveraging GSFC expertise developing spaceflight analytical protocols and instrumentation, we are adapting existing protoflight instrumentation to analyze small volumes of non-aqueous lipid extracts. We will (1) conduct a side-by-side comparison of the state-of-the-art analytical flight technique (SAM-like thermochemolysis) of a raw planetary analog sample and an ExCALiBR lipid extract coupled to a gas chromatography mass spectrometer (GC-MS), and (2) couple the PICASSO-developed Molecular Analyzer for Complex Refractory Organics-rich Surfaces (MACROS) breadboard with ExCALiBR. This laser desorption/ionization-mass spectrometer (LD-MS) will enable analysis of complex and polar organic molecules that are extracted, processed, and concentrated by ExCALiBR, and is particularly relevant for lunar samples.

Machine Learning Development: Sample processing sequences for lipid extraction are complex and consumable-intensive; parameters vary depending on classes of lipids present. We are using machine learning to determine resource-efficient processing sequences to maximize extraction yield. Laboratory scientists use training and experience to make sample analysis decisions; we utilize this logic (*compiled from experienced biogeochemists at Ames, Goddard, and Shell*) on how data collected from the first sample informs subsequent decisions to build a sample processing selection policy (*e.g. solvent selection, step order, subsequent analytical processes applied*).

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