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Forum Article

Future of the Search for Life: Workshop Report

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Abstract

The 2-week, virtual Future of the Search for Life science and engineering workshop brought together more than 100 scientists, engineers, and technologists in March and April 2022 to provide their expert opinion on the interconnections between life-detection science and technology. Participants identified the advances in measurement and sampling technologies they believed to be necessary to perform *in situ* searches for life elsewhere in our Solar System, 20 years or more in the future. Among suggested measurements for these searches, those pertaining to three potential indicators of life termed ''dynamic disequilibrium,'' ''catalysis,'' and ''informational polymers'' were identified as particularly promising avenues for further exploration. For these three indicators, small breakout groups of participants identified measurement needs and knowledge gaps, along with corresponding constraints on sample handling (acquisition and processing) approaches for a variety of environments on Enceladus, Europa, Mars, and Titan. Despite the diversity of these environments, sample processing approaches all tend to be more complex than those that have been implemented on missions or envisioned for mission concepts to date. The approaches considered by workshop breakout groups progress from nondestructive to destructive measurement techniques, and most involve the need for fluid (especially

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liquid) sample processing. Sample processing needs were identified as technology gaps. These gaps include technology and associated sampling strategies that allow the preservation of the thermal, mechanical, and chemical integrity of the samples upon acquisition; and to optimize the sample information obtained by operating suites of instruments on common samples. Crucially, the interplay between science-driven life-detection strategies and their technological implementation highlights the need for an unprecedented level of payload integration and extensive collaboration between scientists and engineers, starting from concept formulation through mission deployment of life-detection instruments and sample processing systems. Key Words: Search for life—Sample handling—*In situ* measurement—Informational polymer—Dynamic disequilibrium—Catalysis. Astrobiology 24, 114–129.

1. Introduction

1.1. Future of the Search for Life workshop goals

The Future of the Search for Life (FoSL) science and engineering workshop, hosted by the Network for Life Detection research coordination network, was held virtually, in two parts, during the spring of 2022. Cosponsored by the NASA Planetary Exploration Science Technology Office and the NASA Astrobiology Program, the workshop was designed to gather current feedback from experts in relevant fields on the interconnections between life-detection science and technology. The overarching goal of the workshop was to promote discussion between scientists and engineers to foster a better understanding of the perspectives and constraints within each discipline and to collectively identify the needs for technologies to perform *in situ* searches for life elsewhere in our Solar System, 20 years or more in the future.

Topics explored during the workshop included what biosignatures to search for, how to carry out that search at Enceladus, Europa, Mars and Titan, and what technologies would be needed for this search. The workshop did not involve detailed discussion of the interpretation of a collection of search-for-life measurements to assess the confidence in their outcome; this was the goal of the 2021 Standards of Evidence workshop (Meadows *et al.*, 2022).

To achieve the above goals and address these topics, the workshop was planned to be highly participatory and interactive with broad participation from academic, commercial, and government professionals across the science and engineering communities, including instrument scientists (see the Acknowledgments section for avenues of advertisement). More than 350 workshop applications were received with workshop participation limited to 100 due to logistical constraints. Participant career stages spanned from graduate students to senior career scientists and engineers, one-third of whom self-identified as primarily engineers and the other two-thirds as primarily scientists (Fig. 1). To foster new ideas and participation outside of the traditional life-detection community, attendance by NASA-center scientists and engineers was capped at 30%. Applicants were asked to describe science and/or engineering experiences and interests that they thought might be relevant to the workshop; applicants with similar expertise or professional backgrounds were selected based on their answer to this question.

The constraint of synchronous activities led to the preferential selection of applicants from time zones in which the workshop sessions (12:30 to 16:30 US Eastern time) took place during work hours.

1.2. FoSL workshop structure

The workshop was held virtually (online) due to the COVID-19 pandemic. It used the structure of a NASA Science Traceability Matrix (STM; Fig. 2) as a framework to define life-detection science objectives and identify corresponding measurement needs. To prepare for the workshop, participants—experts in their field, but most by design without direct science mission planning and implementation experience—were asked to view an *Introduction to the Science Traceability Matrix,* a presentation given by Leisner (2021), to review the STMs developed for the Europa Lander (Hand *et al.*, 2017) and the Enceladus Orbilander (MacKenzie *et al.*, 2020) life detection mission concepts, and to become familiarized with previously considered types of signs of life (Neveu *et al.*, 2018).

The use of an STM framework led the workshop to be separated into two parts (Table 1). Week 1 (March 21–25, 2022) was focused on life-detection science objectives with

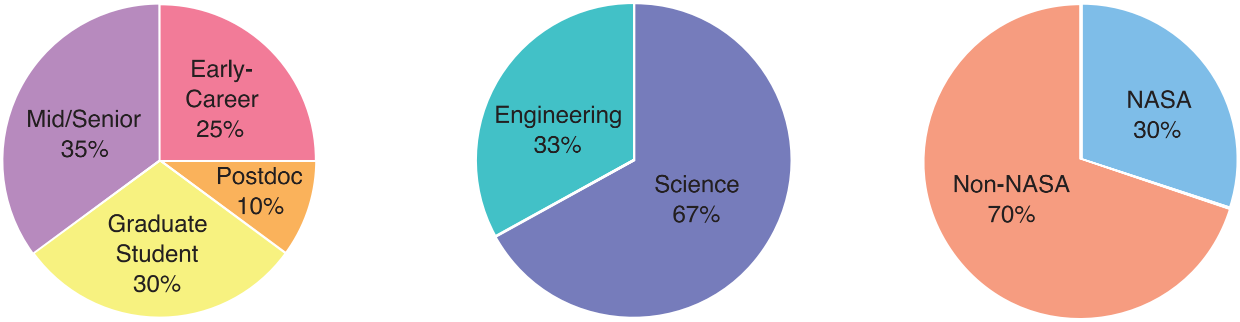

FIG. 1. Professional distribution of FoSL participants based on information entered in participant applications. ''Early career'' implies being within about 10 years of receiving one's terminal degree; ''mid/senior career'' implies over 10 years; participants self-categorized their career stage. FoSL = Future of the Search for Life.

a

Science Goals	Science Objectives	Scientific Measurement Requirements		Instrument Requirements		Projected Performance	Mission Requirements (Top Level)
		Physical parameters	Observables				
		Column Density of Absorber	Absorption Line	Alt. Range	XX km	ZZ km	Observing strategies: requires yaw and elevation maneuvers
		Density and Temperature of Emitter	Emission Line				Launch window: to meet nadir and limb overlap requirement. Window applies day -to-day.

b

Column #	1	2	3	4	5	6	7	8
Column	Science Goals	Science Objectives	Measurement Requirements		Instrument functional requirements		Sample handling requirements (not included in standard STM template)	Top-level mission requirements
			Physical parameters	*Observables*	*Required performance*	*Projected performance*		
Type of information	Science goals are broad and must be identified by NASA as "high value," as established by relevant quotes from NASA and National documents	Science Objectives are specific and capable of being validated. Strongly phrased objectives start with fundamental science questions and turn them into testable hypothesis-driven predictions	Physical parameters of the body under investigation. Quantify how well those parameters need to be determined to meet science objectives: • Spatial coverage • Spatial resolution • Detection limits • Measurement accuracies	Measured observables that will be used to determine / infer physical parameters of the body under investigation	• Signal intensity, dynamic range, sensitivity • Spectral bandwidth and resolution • Field of view • Other instrument-specific metrics	Instrument capability (Current Best Estimate); performance margin is the difference between capability and requirement	Sample processing: • Sample state including key phase properties (e.g., pH, grain size, partial pressure) • Sample size(s) through preparation step(s) (e.g., splitting, combination, reuse) • Phase / temperature through preparation/ preservation step(s) (e.g., melting, heating) with associated timing, duration • Contamination (particulate, chemical, microbial) • Cross-contamination. Sample acquisition: • Sample size range, accuracy, precision • Number of samples • Sample location relative to spacecraft: range, accuracy, precision • Cross-contamination • Forward and, if applicable, backward contamination (particulate, chemical, microbial).	Mission aspects driven by the science (e.g., not the payload mass and power): • Get the instrument to the place it needs to be to conduct the experiment • Operate the instrument for the experiment duration • Get the data back to the scientists

FIG. 2. The FoSL workshop used the NASA STM, taken from NASA's Announcement of Opportunity template (SOMA, 2018), as a structural framework (subset of rows shown in panel **a**). Week 1 was focused on Science Objectives and Scientific Measurement Requirements (columns 2–4). Week 2 was focused on quantification of these measurement needs to inform columns 5–6. However, specification of instrument requirements was beyond the scope of the workshop. Sample handling needs were also considered during week 2 (**b;** column 7). The guidance provided to participants on STM input was based on NASA PI Launchpad presentations by Feldman (2019), Pugel (2021), and Leisner (2021), available from https://science.nasa.gov/researchers/pi-launchpad-sessions. STM = Science Traceability Matrix.

STM flow-down through scientific measurement requirements, including the definition of measurement physical parameters and observables at a broad level. These measurement needs were refined through asynchronous work between the two workshop weeks. Week 2 (April 11–15, 2022) was focused on quantifying the measurement needs and defining mission and instrument-specific sample-handling needs.

To maximize participation and exchange of ideas, each day of the workshop included a balance of plenary (talks and small-group reports) and small-group activities. Two stages of breakout groups were formed. On days 1–3, 15 small groups of 4–7 people were formed to facilitate participation in discussions aimed to lead to the emergence of new ideas (Fig. 3). From day 4 onward, 8 larger groups of up to 9–12 people, with a breadth of expertise within each group relevant to the planetary environment investigated, were focused on potential Solar System exploration environments (*e.g.,* Mars' caves, ocean world plume, Europa ocean), as described in Section 3. Groups were formed based on participant exploration-environment preferences, and to balance demographics (Fig. 1).

During both stages, participants remained in the same group, although occasionally participants helped other groups if their expertise was needed. The eight members of the scientific organizing committee assisted the groups, as needed, in working effectively.

2. Life-Detection Mission Science

2.1. Identifying indicators of life: beyond the state of the art

A workshop goal was to define potential search-for-life approaches for implementation 20 years or more in the

Table 1. High-Level Schedule of Future of the Search for Life Workshop Activities

Day	*Theme*	*Presentations*	*Breakout session tasks+report out*
1	Beyond the state of the art	Europa Lander and Enceladus Orbilander STMs	Address the question: ''What should we look for?'' (15 groups)
2	Seek the full diversity of signs of life	Life Detection Forum and LDKB taxonomy	Categorize and broaden the output of Breakout 1 using LDKB Taxonomy: Chemistry/Structure/Activity. Identify sets of signs of life that provide complementary information. (15 groups)
3	Hypotheses and information needed	Exploration environments (Mars, subsurface oceans, Titan), Earth analogs, agnostic signatures	Identify the information needed to characterize indicators identified in Breakout 2. (15 groups)
4	Destinations and environments	NASA Innovative Advanced Concepts: Titan submarine, Enceladus vent explorer, Sensing with Independent Micro-swimmers, Mars borebots, Bioinspired Ray for Extreme Environments and Zonal Exploration	Based on the list of indicators of life and information needed compiled from Breakouts 1–3 (Fig. 3), choose, prioritize, and reconsider items for the group's exploration environment. (8 groups)
5	Week 1 STM synthesis	Refresher on STM measurement requirements	Formulate quantitative objectives, measurement parameters, and observables based on Breakout 4 outcomes. (8 groups)
Intermission—solidify traceability down to measurement needs, focusing on hitherto underdeveloped indicators (*i.e.,* those not considered by state-of-the-art mission concepts such as Europa Lander and Enceladus Orbilander)			
6	Measurement needs for underdeveloped indicators	Review and feedback on traceability tables so far	Determine what is involved in measuring the *dynamic disequilibrium* indicators in the group's exploration environment. (8 groups)
7		Breakout session: Determine what is involved in measuring the *catalysis* indicator in the group's exploration environment. (8 groups)	Determine what is involved in measuring the *informational polymer* indicator in the group's exploration environment. (8 groups)
8	Sample handling	Breakout session: Assess sample acquisition needs for search-for-life measurements in the group's exploration environment. (8 groups)	Assess sample processing needs for measurements in the group's exploration environment. (8 groups)
9	Documentation of measurement and sample handing needs and their rationales	Framework of this report	Document measurement and sample handling needs and rationales. (8 groups)
10		Breakout session: report figures and tables	Finalize documentation of measurement and sample handling needs and rationales. (8 groups)

Days 1–5 took place during March 21–25, 2022. After a 2-week intermission, days 6–10 took place during April 11–15, 2022.
LDKB = Life-Detection Knowledge Base; STM = Science Traceability Matrix.

future. To set the stage to move beyond the current state of the art during workshop breakout sessions, presentations were given on the first day on the science traceability of the state-of-the-art mission concepts Europa Lander (Alison Murray, Desert Research Institute) and Enceladus Orbilander (Shannon MacKenzie, Johns Hopkins University Applied Physics Laboratory). From these presentations, and their underlying mission concept studies (Hand *et al.*, 2017, 2022; MacKenzie *et al.*, 2020, 2022), specific commonalities in life-detection approaches were identified, including the following:

- Characterization of sample organic content: (1) molecular weight distributions; (2) identification of amino acids and measurement of relative abundances and enantiomeric ratios; (3) identification of lipids and measurement of relative abundances; (4) measurement of carbon stable isotopes.
- Identification of microscale morphological features indicative of cellular organization.

Asked to move beyond these current approaches and signatures, 15 breakout groups (Table 2) were formed and asked to consider, in broad terms, the question ''What should we search for?'' The groups were also asked to evaluate potential sources of uncertainty for each indicator identified, to address the question ''How definitive is the indicator?'' Following this brainstorming session, the groups were asked to categorize their ideas to facilitate comparison and discussion across breakout groups.

2.2. Categorizing indicators: the diversity of signs of life

To provide a basis for categorizing indicators of life, participants were introduced to an ongoing parallel activity, the Life Detection Forum (LDF) by Tori Hoehler (NASA Ames Research Center). The LDF is a ''live'' web-based, community-driven suite of tools established to centralize and organize the body of knowledge needed to support

Life-Detection Category: Chemistry															
Search for...	Breakout Group														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Molecular Structure	■	■			■	■	■	■	■	■	■	■	■	■	■
Abundance and Distribution of Compounds			■		■			■	■	■	■	■		■	
Enantiomer Ratios			■		■	■	■	■	■		■	■	■		■
Elemental Ratios					■							■			
Isotope Ratio Patterns					■			■	■	■	■	■	■	■	■
Mineral Compositions										■				■	■
Chemical Properties					■			■			■				

Life-Detection Category: Structure															
Search for...	Breakout Group Number														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Cellular Morphology	■	■	■		■	■	■	■	■	■	■	■			■
Non-Cellular Morphologies/Textures	■							▒					■		

Life-Detection Category: Activity (Dynamic Disequilibrium)															
Search for...	Breakout Group Number														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Reproduction					■			■	■						
Growth					■			■	■	■					
Seasonality/Temporal Changes		■	■					■	■						
Motion	■				■	■		■	■	■		■			
Catalysis				■			■						■		
Metabolism	■							■			■				
Chemical Selection (temporal and spatial)				■											
Darwinian Evolution					■										
Physicochemical Fluxes								■						■	■

FIG. 3. Indicators of life identified by the workshop participants depicted by breakout group.

program planning, mission concept and technology development, and interpretation of findings.

The LDF includes the Life Detection Knowledge Base (LDKB) (https://lifedetectionforum.com/ldkb), which is designed to organize objects, patterns, and processes that might provide evidence for life. For each such potential piece of evidence, information is presented on the likelihood of false-positive (abiotic prevalence and feature strength) and false-negative (biological prevalence and feature strength) interpretations in a given environmental context, using arguments and evidence from the scientific literature that support or contradict each hypothesis. The LDKB thus centralizes and streamlines the diverse, diffuse, and multidisciplinary astrobiology knowledge of indicators of life.

The LDF is being designed to also include a tool to analyze existing and emerging capabilities to observe these indicators of life. Together, the LDF tools allow the establishment of science traceability from life-detection science objectives to science measurement and instrument requirements. These tools also help identify knowledge gaps and assess mission science risk.

To encourage participants to think more broadly than indicators of life sought by state-of-the-art mission concepts (Hand *et al.*, 2017, 2022; MacKenzie *et al.*, 2020, 2022), a presentation was given on the taxonomy of the LDKB by Davila *et al.* (in preparation) (NASA Ames Research Center). The LDKB taxonomy was developed to incorporate fundamental traits of life as we know it that are related to chemistry, structure, and activity and are broadly acknowledged by the science community. In addition, the framework is based on the underlying principle that it should not have an inherent hierarchy that might lead to perceived or unconscious bias (*e.g.,* regarding the technical feasibility of detection).

The LDKB taxonomy was also structured to provide the flexibility to incorporate new knowledge as it is developed, and with sufficient granularity to allow adequate and comparable level of detail to provide a basis for comparison and evaluation. Within each of the Chemistry, Structure, and Activity categories, the taxonomy includes potential biosignatures, which are physical or chemical properties, or their time-dependent changes, which could potentially reveal the presence of life. Within the LDKB, a potential biosignature could have an outcome that supports the presence of life (a positive or false-positive interpretation) or an outcome that is inconsistent with the presence of life (a negative or false-negative interpretation).

A compilation of indicators of life identified by the 15 groups of participants and categorized by topic is shown in Fig. 3. While many of these indicator types have been considered extensively in the development of past mission concepts, others were comparatively novel and understudied. From among this second group, the workshop organizers

Table 2. The 15 Breakout Groups That Addressed the Question "What Indicators of Life Should We Search for?" and Evaluated Potential Sources of Uncertainty to Address the Question "How Definitive Is the Indicator?"

Group 1	*Group 2*	*Group 3*
Frances Bryson (Georgia Tech) Bryana Henderson (NASA JPL) Sayali Mulay (U. Tenn. Knoxville) Mike Padgen (NASA ARC)	Kathryn Bywaters (Honeybee Robotics) Maria Carrillo (Wichita State U.) Erin Leonard (NASA JPL) Alison Murray (Desert Res. Inst.) Peter Schroedl (Boston U.) Yi-Qiao Song (Harvard U.)	Chris Lindensmith (NASA JPL) Jingjun Liu (Yale U.) Melissa Trainer (NASA GSFC) Marina Walther-Antonio (Mayo Clinic) Ziming Yang (Oakland U.)
Group 4	*Group 5*	*Group 6*
Eve Berger (Texas State U.) Madeleine Bodine (U. South Carolina) Francesca Cary (U. Hawai'i) Keyron Hickman-Lewis (UK Natural History Museum) Pavel Klier (NASA ARC) Alvin Yew (NASA GSFC)	Nathalie Cabrol (SETI Institute) Seán Jordan (IST, Lisbon) Gordon Love (UC Riverside) Chinmayee Govinda Raj (Georgia Tech) Vishaal Singh (Columbia U.) Elizabeth Spiers (Georgia Tech)	Lu Chou (NASA GSFC) Lucas Fifer (U. Washington) Jessica Koehne (NASA ARC) Andrew Patrick (Lighthouse Lab Serv.) Nicholas Speller (Georgia Tech) Tessa Van Volkenburg (JHU/APL)
Group 7	*Group 8*	*Group 9*
Andrew Gangidine (Cranbrook Inst.) Heather Graham (NASA GSFC) Hemani Kalucha (Caltech) Brook Nunn (U. Washington) Tony Ricco (NASA ARC)	Aaron Burton (NASA JSC) Andrea Corpolongo (U. Cincinnati) Craig Herbold (U. Vienna) Andy Mullen (Cornell U.) Alex Walker (Sierra Lobo, Inc.)	Nathan Bramall (Leiden Meas. Tech.) Diana Gentry (NASA ARC) Patrick McNally (U. Michigan) Taylor Plattner (Georgia Tech) Sawsan Wehbi (U. Arizona) Peter Willis (NASA JPL)
Group 10	*Group 11*	*Group 12*
Kae Aithinne (JHU/APL) Desiree Baker (U. Cincinnati) Jungkyu (Jay) Kim (U. Utah) Neveda Naz (Tufts U.) Noah Tashbook (Caltech)	Morgan Cable (NASA JPL) Zaid Haddadin (UC San Diego) An Li (U. Washington) Erik Long (Orbotic Systems, Inc.) Kristian Persson (SwRI) Svetlana Shkolyar (NASA GSFC/U. MD) Jennifer Timm (Rutgers U.)	Evan Eshelman (Impossible Sensing) Mihaela Glamoclija (Rutgers U.) Jian Gong (MIT) Maëva Millan (CNRS/LATMOS) Vinitra Nathan (Dartmouth College) Michael Tuite (NASA JPL)
Group 13	*Group 14*	*Group 15*
Marissa Cameron (NASA JPL) Christos Georgiou (U. Patras) Carolynn Harris (Dartmouth College) Aila Inaba (Rutgers U.) Shannon MacKenzie (JHU/APL) Aaron Regberg (NASA JSC)	Liliane Burkhard (U. Hawai'i) Milton Cordeiro (NASA ARC) Kas Knicely (U. Alaska) Kennda Lynch (Lunar Pl. Inst.)	Anna Butterworth (UC Berkeley) Mostafa Hassanalian (New Mexico Tech) Jordan McKaig (Georgia Tech) Grace Ni (U. Maryland) Lucien Weiss (Polytechnique Montreal)

U. = university.

identified *dynamic disequilibrium*, *catalysis*, and *informational heteropolymers* (referred to as "informational polymers" in the rest of this report) as particularly promising avenues for further exploration during week 2 of the workshop. As shown in Fig. 3, "dynamic disequilibrium" encompasses the concept of spatial and temporal variations in fields of physicochemical properties that are inconsistent with those of an abiotic system. "Catalysis," a subset of dynamic disequilibrium, rests on the idea that life can hasten otherwise slow or improbable chemical reactions.

The search for informational polymers is routinely carried out on Earth and was included in the Enceladus Orbilander concept's instrument payload, but with a recognized low degree of technical maturity at this time (MacKenzie *et al.*, 2021).

In addition to identifying indicators of life, participants were asked to consider which combinations of indicators would facilitate the assessment of the biological or abiotic origin of individual indicators. This informed the choice of measurement needs for different exploration environments, discussed in Section 3.

3. Science Traceability of Future Search-for-Life Mission Concepts

3.1. Science scope and measurement needs at exploration environments

Having collectively determined the breadth of possible signs of life that future missions could search for (Fig. 3), the workshop focus shifted to exploration environments for this search to take place within. During days 3–4, participants voted to form groups investigating the science traceability of concepts exploring a specific environment. Two sets of five plenary talks focused on (1) the science of these candidate environments and (2) advanced engineering solutions that could enable exploration there (Table 1).

Science presentations focused on the search for life on Mars: present life, recent life, ancient life (Chris McKay, NASA Ames Research Center); subsurface oceans (Chris Glein, Southwest Research Institute); life in ice (Jill Mikucki, Univ. Tennessee, Knoxville); the science of the Dragonfly mission (Melissa Trainer, NASA Goddard Space Flight Center); and agnostic signatures of life (Sarah Johnson, Georgetown University). Engineering presentations all focused on projects funded by the NASA Innovative Advanced Concepts program: Titan Submarine: Exploring the Depths of Kraken Mare (Steven Oleson, NASA Glenn Research Center), Enceladus Vent Explorer (Masahiro Ono, Jet Propulsion Laboratory), Sensing with Independent Micro-swimmers (Ethan Schaler, Jet Propulsion Laboratory), Borebots: Tetherless Deep Drilling into the Mars South Polar Layered Deposits (Quinn Morley, Planet Enterprises), and Bioinspired Ray for Extreme Environments and Zonal Exploration (Javid Bayandor, SUNY Buffalo).

Participant votes (first, second, and third choice were expressed) resulted in the formation of eight exploration environment groups: Ocean World Plume, Europa Ocean, Europa Ice Shell, Mars (1; Open Cave), Mars (2; Subsurface, several meters depth), Enceladus Near-Surface Ice (vent and upper ice shell), Enceladus Ocean including ice and rock interfaces, and Titan Sea (Table 3). Other destinations or types of environments were considered, including Venus' atmosphere and interiors of ice giant moons or dwarf planets, but did not gather sufficient participant support to warrant the formation of dedicated breakout groups.

Table 3. The Eight Groups That Identified Measurement and Sample Handling Needs, Including Those Shown in Table 4, at Environments of Enceladus, Europa, Mars, and Titan

Enceladus near-surface ice	*Enceladus ocean and interfaces*	*Ocean world plume*
Nathan Bramall (Leiden Meas. Tech.)	Jessica Koehne (NASA ARC)	Kae Aithinne (JHU/APL)
Morgan Cable (NASA JPL)	Chris Lindensmith (NASA JPL)	Anna Butterworth (UC Berkeley)
Marissa Cameron (NASA JPL)	Erik Long (Orbotic Systems, Inc.)	Nathalie Cabrol (SETI Inst.)
Andrea Corpolongo (U. Cincinnati)	Kennda Lynch (Lunar Pl. Inst.)	Lucas Fifer (U. Washington)
Jian Gong (MIT)	Shannon MacKenzie (JHU/APL)	Craig Herbold (U. Vienna)
Zaid Haddadin (UC San Diego)	Vinitra Nathan (Dartmouth College)	Aila Inaba (Rutgers U.)
Jungkyu (Jay) Kim (U. Utah)	Brook Nunn (U. Washington)	Jordan McKaig (Georgia Tech)
Maëva Millan (CNRS/LATMOS)	Mike Padgen (NASA ARC)	Patrick McNally (U. Michigan)
Yi-Qiao Song (Harvard U.)	Elizabeth Spiers (Georgia Tech)	Grace Ni (U. Maryland)
	Noah Tashbook (Caltech)	
	Sawsan Wehbi (U. Arizona)	
	Ziming Yang (Oakland U.)	
Europa ice shell	*Europa ocean*	*Titan sea*
Madeleine Bodine (U. South Carolina)	Desiree Baker (U. Cincinnati)	Frances Bryson (Georgia Tech)
Liliane Burkhard (U. Hawai'i)	Eve Berger (Texas State U.)	Francesca Cary (U. Hawai'i)
Kathryn Bywaters (Honeybee Robotics)	Maria Carrillo (Wichita State U.)	Lu Chou (NASA GSFC)
Evan Eshelman (Impossible Sensing)	Diana Gentry (NASA ARC)	Mostafa Hassanalian (New Mexico Tech)
Mihaela Glamoclija (Rutgers U.)	Kas Knicely (U. Alaska)	Hemani Kalucha (Caltech)
Bryana Henderson (NASA JPL)	Andy Mullen (Cornell U.)	Erin Leonard (NASA JPL)
Pavel Klier (NASA ARC)	Jennifer Timm (Rutgers U.)	Kristian Persson (SwRI)
Alison Murray (Desert Res. Inst.)	Melissa Trainer (NASA GSFC)	Taylor Plattner (Georgia Tech)
Neveda Naz (Tufts U.)	Tessa Van Volkenburg (JHU/APL)	Marina Walther-Antonio (Mayo Clinic)
Chinmayee Govinda Raj (Georgia Tech)		Lucien Weiss (Polytechnique Montreal)
Peter Willis (NASA JPL)		Alvin Yew (NASA GSFC)
Mars 1: open cave	*Mars 2: subsurface*	
Aaron Burton (NASA JSC)	Andrew Gangidine (Cranbrook Inst.)	
Emily Cardarelli (NASA JPL)	Christos Georgiou (U. Patras)	
Milton Cordeiro (NASA ARC)	Heather Graham (NASA GSFC)	
An Li (U. Washington)	Carolynn Harris (Dartmouth College)	
Andrew Patrick (Lighthouse Lab Serv.)	Keyron Hickman-Lewis (UK Nat. His. M.)	
Tony Ricco (NASA ARC)	Seán Jordan (IST, Lisbon)	
Peter Schroedl (Boston U.)	Jingjun Liu (Yale U.)	
Svetlana Shkolyar (NASA GSFC/U. MD)	Gordon Love (UC Riverside)	
Nicholas Speller (Georgia Tech)	Sayali Mulay (U. Tenn. Knoxville)	
Michael Tuite (NASA JPL)	Aaron Regberg (NASA JSC)	

This relative lack of support may be because until these environments are better characterized, the prime astrobiological focus is on assessing their habitability rather than searching for signs of life.

From day 4 onward, each group developed the science traceability, moving left to right, starting with the science objectives defined for their environment and stopping before instrument requirements. Although not explicitly included in the current NASA STM template, sample handling was also considered (Fig. 2).

This involved first defining the scope of the search-for-life science at their destination environment. Given the limited time, contextual measurements, including those characterizing habitability, were deemed out of scope. Groups were further asked to select a limited set of indicators of life from the list in Fig. 3 that they considered best able to obtain a meaningful mission outcome. During day 5 and in the intermission between the two workshop weeks, groups focused on crafting quantitative measurement needs (physical parameters and observables) for each of their selected indicators. Finally, the first 2 days of week 2 were dedicated to developing measurement needs for indicators within the three understudied types identified above: dynamic disequilibrium in general, catalysis in particular, and informational polymers. Groups considered how the environment affects their measurement, and rationalized quantitative aspects of their measurement needs in terms of their potential for distinguishing biological from abiotic sources.

Developing formal measurement requirements normally takes a dedicated mission concept study team several months. Therefore, the product of this exercise, of which a composite summarized version is provided in Table 4 with emphasis on understudied indicators, should not be taken as definitive but rather as an indicative source of inspirational concept suggestions for future mission development efforts. Some of the objectives and associated measurements shown in Table 4 were intended by breakout groups to be paired with objectives previously considered for existing mission concepts (and as such not reported here for brevity). For example, the objective ''Determine the temporal changes of chemical complexity within the Titan Lake environment'' (row #17; formulated by the Titan Sea group) was paired with another objective ''Quantify the intrinsic chemical complexity of molecules'' not shown in Table 4 because it is one of the objectives of the Enceladus Orbilander mission concept (MacKenzie *et al.*, 2022). Objectives and measurement needs are associated with the group(s) that defined them, but many are relevant to other planetary environments as well.

Identified informational polymer measurements tend to focus on characterizing these polymers in terms of physical properties (*e.g.,* size), chemical properties (*e.g.,* reactivity to, and reversibility of, assembly or modification reactions), and informational or functional properties (*e.g.,* encoding system, ability to fold). Identified catalysis measurements focused on rates for, and by-products of, classes of reactions such as hydrolysis, and on the presence of known catalysts such as organometallic compounds. Other identified dynamic disequilibrium measurements involve changes to environmental organic and inorganic chemistry, changes in macroscale morphology, and microscale particle or molecular motion.

For each of the measurement needs shown in Table 4, a rationale based on the literature or on experience was provided. These are provided in the Supplementary Data. As one example of the detailed information captured, the rationale for the measurement addressing the objective ''Characterize physicochemical fluxes/gradients that are against thermodynamics or abiotic conditions'' (row #7) is as follows:

- Spatial range informed by biofilm thicknesses of 30–400 μm (Murga *et al.*, 1995).
- Spatial resolution informed by changes in metabolic activity (*e.g.*, nitrate/nitrite utilization) can be observed vertically stratified at μm scales. In a 120-μm-thick biofilm exposed to an oxic environment, the O_2 concentration approaches 0 at 60 μm depth (Stewart *et al.*, 2019).
- Concentrations: Glucose-fed *Escherichia coli* cells contain millimolar concentrations of amino acids (especially glutamic acid), redox molecules (*e.g.*, glutathione), nucleotide triphosphates, glycolytic pathway intermediates (*e.g.*, fructose-1,6-bisphosphate), and electron transfer cofactors (*e.g.*, NAD^+/NADH) (Bennett *et al.*, 2009).
- Uranium can be used as a redox indicator (Romaniello *et al.*, 2013).

As a second example, the rationale provided for the measurement addressing the objective ''Search for evidence of catalysis by ≥1 microorganism'' (row #9) states the following:

- Hydrolysis reactions are targeted in the search for catalytic activity because:
    - they are mostly exergonic (do not require an unknown form of energy source akin to adenosine triphosphate on Earth) (Georgiou, 2018)
    - they involve the largest (200) and most diverse of the 6 main classes of enzymes (Shukla *et al.*, 2022).
- Testing for hydrolytic catalytic activity: incubate with known artificial substrates that can be catalytically broken into known products, including a fluorophore or chromogene, by specific hydrolytic catalytic activity (Georgiou, 2018).
- Candidate products:
    - fluorogenic and/or absorbing ultraviolet or visible radiation, based on a periodate ($NaIO_4$)-coupled β-elimination of umbelliferone (Badalassi *et al.*, 2000) and p-nitrophenol (Beisson *et al.*, 2000)
    - chromogenic indirect assays, such as the back-titration method with adrenaline (Fluxá *et al.*, 2008).
    - Detailed lists of artificial substrates are provided in Badalassi *et al.* (2000) and Reymond (2008) and references therein.
- Limit of detection (LoD): Fluorophore LoD = 0.5 p*M* (for fluorescein); chromogenic product = typical absorbance instrument (0.005 A), which can be miniaturized for flight using, for example, optofluidics (Yin *et al.*, 2006).
- Sample needed: Cell protein content $\geq 4 \times 10^{-15}$ g, 55% of *E. coli* dry mass (Milo, 2013; Zotter *et al.*, 2017). Single-cell-scale detection methods provided by Di Carlo *et al.* (2006), Kovarik and Allbritton (2011), and Zotter *et al.* (2017).
- Substrate concentrations in Earth cells: 1–100 μ*M* (Albe *et al.*, 1990; Zotter *et al.*, 2017).

TABLE 4. SUMMARY OF BREAKOUT-GROUP SCIENCE TRACEABILITY CONCEPTS FOR THE *IN SITU* SEARCH FOR LIFE AT VARIOUS SOLAR SYSTEM ENVIRONMENTS BEYOND EARTH

Environment	Science objectives	Understudied indicator	Measurement needs	Example sample handling needs
OW plume	Search for proteins in the plume and determine their sequence	Other	• Measure proteins and protein metabolites and their sequences at >1 nM • Measure the sequence and abundance of amino acid monomers • Identify modifications to the polymer (e.g., phosphorylation)	Remove or reduce interfering inorganic ions, typically to <1 mM
OW plume; Europa Ocean	Search for DNA or other charged linear polymers	Informational polymers	For polymers ≥ 20–7000 monomers in length ≥ parts-per-quadrillion by mass, determine: • Length and diameter • Consistent "backbone" (molecule which can polymerize) • Changing subunits • Hydrodynamic radius • Surface charge (ζ potential) and its variation within the polymer • Any higher-level structure (e.g., folding resulting in surface functionality).	• Sample size: 1-100 mL • Sample state: liquid • Lyse any cells • Remove or reduce interfering inorganic ions, typically to <1 mM • Purify by charge or stickiness • Neutralize if pH is not between 4 and 10
Europa Ocean	Search for evidence of cell-like activity	Dynamic disequilibrium	Determine variations in space and/or time of: • Density ($kg\ m^{-3}$) • Refractive index	Sample state: liquid
Europa ice		Dynamic disequilibrium	Identify particles at scales ≥ 0.1 μm with a mean square displacement changing at a rate not equal to the rate of change of time within 3 standard deviations	• Sample volume: $10\ cm^3$ • Sample state: solid • Vertical separation between samples: 10 cm • Collect triplicate samples to a depth of 2 m • Preserve chemical and physical context (pH, salinity, dissolved gases, temperature, etc.). • Desalt to < 1 mM
Europa ice		Dynamic disequilibrium	Molecular motion (translocation of molecules and energy): • Static particle boundary polarization to gradients magnitude >30 mV • Particle boundary potential transients that resolve to initial baseline to within 10 mV • Spatially resolved oxidants and reductants to 100 nm resolution	
Mars 1, Mars 2	Search for information polymers	Informational polymers	Information content of polymers > X monomers in length. • diversity of monomer library / number of discrete information-bearing subunits (*i.e.*, base 4, base 20, etc.) • sites for reversible binding to transfer information • conserved motifs / consensus sequences (frequently found) • self-assembly of monomers vs. requirement for driving mechanism • frequency of a building block in a polymer relative to the environment • molecular complexity exceeding abiotic possibilities	• Sample at bottom, top, and side surfaces of an open cave • Spatial spacing: meters • Ability to sample rock, ice, gas, and liquid brine • Record details of gas exchange • Record sample position at the cm scale • Hydrocarbon-clean coring • Preserve spatial integrity, temperature, pressure for initial analyses • Then: - Disaggregate solid samples (e.g., by powdering, melting) - Extract / separate materials from rock hosts - Filter solution / suspensions to remove particles - Concentrate solvent extracts.
Mars 1	Characterize physicochemical fluxes/gradients relative to expected thermodynamics or abiotic conditions	Dynamic disequilibrium	Determine the spatial and temporal distributions of redox potentials. Concentrations over time and distance/space of Na^+, Cl^-, K^+, Ca^{2+}, ammonia, ammonium, nitrates, nitrites, phosphates, CO_2, acetate, lactic acid, ATP, AMP, O_2, sulfur compounds, lipids, and redox-active compounds such as $U^{6+/4+}$ • Accuracy: ~30% for concentrations, 0.1 pH • Spatial range: [Na^+], [Cl^-]: mm to m lateral, 100 μm depth • Spatial positioning accuracy: ±5 μm laterally, ±1 μm depth • Concentration range: [Na^+], [Cl^-]: μM to saturated solution; others: mM to saturation	
Mars 2	Search for and characterize organic bound-transition metals as possible evidence of enzyme cofactors	Catalysis	Search for organometallic molecules and polymers at the ≥ 1 μm scale	• Acquire 1 m long cores (depth profiles) • Depth range: 0 to 5 m • Alteration- and contamination-free • Allow optical measurements of the core during acquisition • Grind and polish subsets of cores • Pulverize, sieve by size fraction, and weigh before analysis • Preserve isotopic composition (e.g., prevent heating)
Mars 2	Search for evidence of catalysis by ≥1 microorganism	Catalysis	Relative rates (product concentration per unit time) of reactions of a sample vs. negative control with an artificial substrate • Measurement duration: ~10 min • Artificial substrate concentration: ~μM • Product LoD: 10 pM	

(*continued*)

TABLE 4. (CONTINUED)

Enceladus surface & vents	Investigate surface and shallow subsurface and/or surfaces of vents, and vent ejecta (fallout), for evidence of biofabrics, e.g. microbial mats, thrombolites, biofilms	N/A	Determine the mineralogical composition and search for organic material • Spatial range: three 1-m^2 fields of view • Spatial resolution: mm scale; μm scale in select organic-bearing regions • Depth: surface and 1 m depth with 1 mm resolution	• Sample at least 3 sites of 1 m^2 coverage each, to 1 m depth, in regions of distinct fallout accumulation rates (≥ 0.1 km apart) • Perform nondestructive measurements → perform destructive measurements → abrade → repeat this cycle • Preserve spatial, thermal, and chemical integrity until nondestructive measurements are complete • Maintain consistent illumination for non-destructive observations.
Enceladus surface & vents		N/A	Map elements C, H, N, O, P, S, Fe, and Ca, co-located with layered or clotted structures • Spatial range: 1 mm^2 per focal point of interest • Spatial resolution: μm • LoD (% dry mass in μm^2 area): C: 1; H: 0.24; N: 0.25; O: 1.625; P: 0.0625; S: 0.025; Fe: 0.0025; Ca: 0.00125	
Enceladus ocean	Constrain the upper limit of possible cellular concentrations in the pelagic environment	N/A	Distribution of particle densities; particle sink or float rate/brownian motion in non-gravity axis <100 particles mL^{-1}, density difference ~0.1 g cm^{-3}	• Pre-sampling mapping in open ocean to home in on areas of interest based on pre-established criteria (*e.g.*, light scattering to find particle-dense areas; T; pH; Eh or their time/spatial gradients in select ranges, *e.g.*, via tracing of Fe oxide particles for redox conditions) • > 40 μL of sample through field of view, ~1 cm^3 total liquid sample per "site" • Preserve: - chemistry (*i.e.*, no introduced molecules) - temperature - within a range depending on the sample (*e.g.*, if melting would invalidate a later measurement) - particle and rock morphologies - mechanical integrity - pressure (for dissolved gas concentrations) • Maintain and log global position, depth, and orientation re: magnetic field, including for time series • Prevent clogging • Controls at every step • Triplicate samples • Filter • Keep track of fluid volume moved in each filter stage to infer original concentrations
Enceladus ocean, Titan sea		Dynamic disequilibrium	Particle motion (non-brownian, non-comoving with flow) with or without stimulus (e.g., substrate addition) • Particle size: 0.1 μm to 1 mm • Spatial range: 1 cm^2 • Velocity resolution: ~1 μm s^{-1} • Velocity range: 1–300 μm s^{-1}	
Enceladus ocean		N/A	Distribution of particle refraction indices N from phase φ shift of transmitted light; $\Delta N \sim 0.1$ ($\Delta\varphi \sim 0.2\pi$)	
Enceladus ocean	Characterize isotopic compositions and fractionation of biologically-relevant elements (C, H, N, O, phosphates, S, Ca, Cl) including complex carbon compounds and their sources	N/A	Isotopic compositions of particles: δD > 10‰ δ^{18}O > 2.6‰ δ^{13}C > 2‰	
Enceladus ocean		N/A	Clumped isotopes measurements of methane and larger organic molecules: $\Delta^{13}CH_3D$ > 0.7‰, $\Delta^{12}CH_2D_2$ > 2‰	
Titan sea	Determine the temporal changes of chemical complexity within the Titan lake environment	Dynamic disequilibrium	• Changes in relative abundance with 10% precision and number of functional groups in molecules • Mass range between 12-1000 Da, with a 1 Da resolution and signal:noise ratio > 10, from a single location to across seasonal transition (< 3 storm events), every 1–10 Titan days	• Sample at spatial increments 10 m; > 3 locations in a 1 cm^2 area • Temporal spacing of sampling is a knowledge gap due to unknown reaction rates at Titan thermal and photochemical conditions; suggest 1-10 Titan days up to 1x/Earth year • Ability to keep instruments/sampling static for several temporal spacings • Reduce spatial/temporal spacings if variability too high • Sample at top, middle, and bottom of lake • Temporal range: seasonal (7 years), second half of winter+first half of spring • Ability to sample solid-liquid interfaces • Preserve spatial distribution and chemical (structural) composition, including noncovalent bonds. Knowledge gaps: temperatures of Titan's lakes (projected to be 91-94 K?), and also the temperature at which weak intermolecular forces and H-bonding break down or get overprinted by covalent-type bonding • Prevent clogging by bubbles or particles • Preserve native temperature within ±2 K, pressure within range (requires further knowledge of chemical reactions on Titan) • Reach ionized form for solid samples of sediment • Keep liquid samples liquid • Ability to filter, add reagents to, and remove methane and ethane from liquids • Limit cross-contamination.
Titan sea	Identify the uptake and release of [labeled] chemical compounds	Catalysis	Isotopic ratio of non-volatile or volatiles of 1 g of sample at 10% precision after addition of isotope-labeled acetylene and H$_2$ • Both before and after adding labeled reagents • 10% precision.	
Titan sea	Characterize and search for changes in nearby shoreline morphology	Dynamic disequilibrium	Repeat morphological and coarse compositional mapping of > 10% of the shoreline > 3x / Titan day • Spatial resolution: 10 cm	
Titan sea	Characterize and search for changes in vibrations within and underneath Titan lakes	Other	• Frequency range: 1–20 Hz • Time resolution: TBD • Duty cycle: 10%	

Within the 20 science objectives and corresponding measurement needs shown, measurement and sample handling needs for understudied indicators of life are emphasized. Full science traceability, including instrument and top-level mission requirements, is not shown as the workshop focused on science rather than measurement techniques. This table is a compilation of concepts developed by individual groups and does not represent all of the suggested search-for-life measurements. A total of ≈ 75 search-for-life measurements were suggested by the breakout groups; those overlapping with published search-for-life STMs (Hand et al., 2017, 2022; MacKenzie et al., 2021) are not shown here. Example sample handling needs may pertain to one or several different samples, and different methods of analysis. Rationales for the measurement and sample handling needs that are shown here are provided in the Supplementary Data.

LoD = limit of detection. N/A = not applicable (indicator has been studied in a different environmental context; see Supplementary Material).

Among the measurements shown in Table 4 that do not pertain to the above-mentioned underdeveloped indicators, the *Ocean World Plume* team suggested sequencing functional polymers (proteins), the group focusing on *Enceladus' Near-Surface Environment* focused on depth profiling of vents for evidence of biofabrics, the *Enceladus Subsurface Ocean* team postulated the feasibility of measuring ratios of organic compound isotopologues (Gilbert, 2021), and the *Titan Sea* team suggested characterizing and searching for changes in vibrations within and underneath Titan's lakes.

3.2. Sample handling

Although not included in the STM template provided in NASA's Announcement of Opportunity (Fig. 2), sample handling is a key consideration of *in situ* search-for-life investigations since *in situ* measurements generally involve both sample acquisition and sample processing before making a measurement. Sample handling constraints arise from the choice of measurement method, which requires there to be a minimum amount of sample in a specific state (*e.g.,* 1 mL of liquid water or 2 g of soil). In turn, sample acquisition needs can drive top-level mission requirements.

A day of the workshop was thus dedicated to sample handling (acquisition and processing). Breakout-group outcomes are reflected in Table 4 and in the example individual sample handling flowcharts shown in Fig. 4.

Despite the wide diversity of destination environments and sampling strategies considered by the eight teams, common needs were identified. These include performing initial reconnaissance at progressively decreasing (nested) scales to home in on the most astrobiologically relevant sampling locations; preserving the mechanical, thermal, and chemical integrity of the samples upon acquisition; optimizing sample consumption by performing the least destructive measurements first in a sequence of analyses; and for liquid samples, filtering and adding reagents.

Salient points noted by several groups pertained to understudied indicators. Measurements of dynamic disequilibrium indicators would tend to require determination of properties along spatial distances and/or at time intervals. For solid samples, this implies preserving the sample's spatial integrity and temperature (to avoid chemical changes), for example, using large drill bits or wide coring devices that affect the inner portions of the sample less significantly, as well as inert fluids such as He or N_2 in gas or liquid form for drilling and polishing. For liquid samples, this would require recording acquisition locations. Measurements of informational polymers would require freeing such polymers from any compartments (*e.g.,* cells) and removing other particulate interference, for example, by filtration.

As was done for measurement needs, each team developed a rationale for sample acquisition and processing needs. Example sample acquisition rationales include, for the Mars 1 breakout group, the following:

- Acquire samples in a cave with full or partial gas exchange with the outside atmosphere and accessible interior on the kilometer scale.
- Why a cave: Reduced radiation exposure, less sample weathering, and narrower operational temperature range. Processes that impact gradients and disequilibria, such as convective transport and large temperature and humidity changes, are likely to be diminished.
- Why open: If access to the cave or void requires drilling, breaching would likely introduce contamination as tailings drop into the void. This would also result in mixing the ambient void atmosphere with the globally mixed atmosphere unless the breach is sealed.
- Key challenges: Communications and mobility within the cave.
- Target thin surface-attached structures that could be biofilms due to the unique geometrical characteristics of films and their potential association with microbial communities. Sample in various gravitational orientations where liquids or ices could collect, stalactites, snottites, or stalagmites be found, or layered structures be seen. This range of sample types distinguishes between extant life (in any type/orientation) and extinct life (likelier in ice or mineral samples). It allows for a higher chance of detection of biomarkers but brings about challenges associated with sampling each sample phase. For example, gasses may indicate a life-related activity, for example, metabolism.
- Spatial integrity preserves distribution information on meter-scale gradients. This lowers the possibility of incorrect interpretations in addition to false negatives and positives.

And for the Titan Sea breakout group:

- Spatial spacing $\approx 0.1\times$scale of geological variation (*e.g.*, if river mouth is approximately kilometer in scale, use a ~ 100 m grid spacing).
- Temporal spacing: 1×/Titan day for daily compositional context and new molecular influxes; every ~10 Titan days to identify specific molecules depending on reaction rates.
- Spatial range: Sufficient to not miss a potential location for life.
- Temporal range: Measure the tail end of the wet winter and start of dry spring to understand:
  - influx of organic molecules from the atmosphere into the lakes
  - how these molecules get physically selected out of the lake
  - how they change over time in the lakes
  - what is left as lakes concentrate
  - what goes back to the atmosphere.

Observing this seasonal change would best capture how the chemical environment of Titan's sea could drive selection processes potentially associated with life's emergence.

An example sample processing rationale (from the Mars 2 breakout group) is as follows:

- Powdering needed for water extraction for both catalysis and sequencing.
- Need to know the particle size distribution to understand mineral phases and put organic matter quantification in context.
- The isotopic composition of elements in both organic and inorganic constituents, relative to that of the bulk reservoir of these elements, is needed to understand the nature of any disequilibrium, both for physical structures and reaction products. Thus, prevent heating

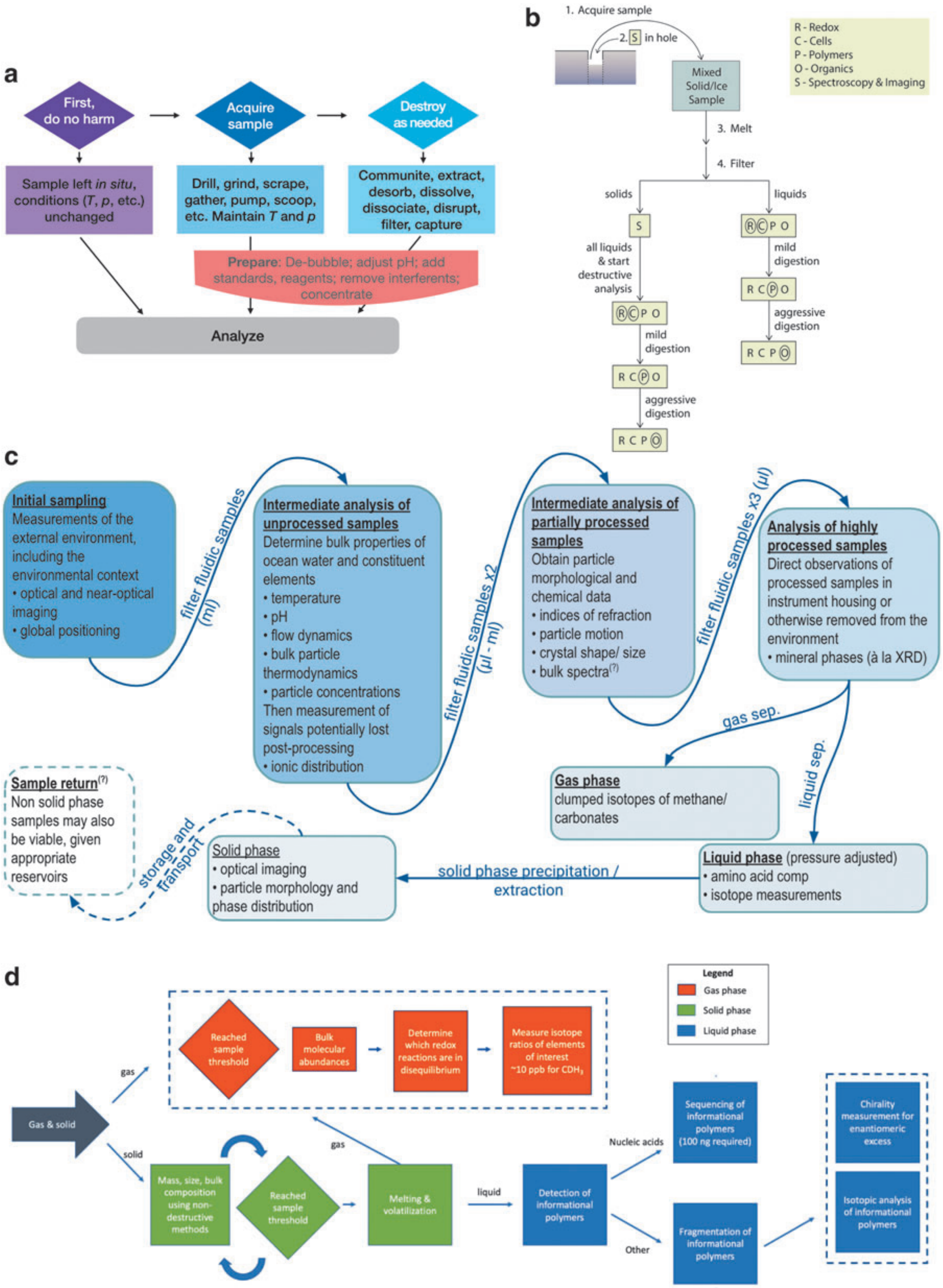

FIG. 4. Collage of sample processing flowcharts developed by individual breakout groups: **(a)** Mars 1—cave, **(b)** Europa Ice Shell (circled letters designate analyses at that stage targeted toward corresponding measurands), **(c)** Enceladus Ocean (extended from Lawrence et al., 2023), and **(d)** Ocean World Plume. Despite the diversity in destination environments, these display surprisingly similar aspects. All tend to be more complex than implemented on or envisioned for mission concepts to date. They progress from least- or nondestructive measurements to the most destructive to make the most of a potentially limited sample, or to obtain a variety of measurements on the same sample. Fluid (in particular liquid) sample is prevalent in the processing chain. The interplay between these science-driven approaches and their technological implementation highlights a need for extensive science–engineering interaction in the upcoming development of sample processing for life detection.

(which may evolve lighter elements) and understand any preferential dissolution or solvation of species that may skew an isotope measurement (*e.g.*, lower solubility of deuterated species in benzene) (Bechalany *et al*., 1989).
- Trace element concentrations associated with organic material are also evidence of disequilibrium. Elemental mapping requires a flat surface, which will require postprocessing of collected drill cores (Gangidine *et al*., 2021) without contaminating the sample in these elements.

While not explicitly part of the sample handling discussion, sample contamination and instrument validation were mentioned by several teams. Introduced Earth materials may not only lead to false positives in search-for-life measurements, but also change local environments, impacting downstream observations. In some cases, measurement methods may involve elements of Earth biology (*e.g., E. coli*-based assays). Approaches to instrument validation could include blanks, negative, and positive controls; they may be specific to each measurement and/or handling step. The value of bringing positive controls from Earth was discussed, but no conclusion was reached.

Overall, the interplay between these science-driven approaches and their technological implementation highlights a need for extensive science–engineering interaction in upcoming development of sample processing for life detection.

4. Potential Technology Directions for the Search for Life 20 Years or More in the Future

For most of the indicators of life identified by workshop participants as targets of search-for-life investigations (Table 4), development of spaceflight instruments able to measure these indicators has been ongoing, and some have flown or been built for flight. However, for a small subset of indicators, there is currently a measurement technology gap. These include the following:

1. Methods and instrument technologies for measuring dynamic disequilibrium (activity). Spaceflight instrument technologies for measurement of time domains have received limited attention. Many existing instrument technologies (*e.g.*, chemical sensors, spectrometers) could potentially be applied to search-for-life strategies based on measurement of activity. For example, the Viking biology experiments used common instrument technologies (*e.g.*, gas chromatography) in an attempt to measure potential biological activity (Klein *et al*., 1976). However, in general, methods, strategies, and instrument packages for measurement of dynamic disequilibrium are undeveloped. Application of current instrument detection technologies in this area will likely require specific technology development, including sample manipulation and processing. In addition, strategies for *in situ* seasonality measurements (*e.g.*, row #19 of Table 4) are lacking, and the value of some indicators such as mechanical vibrations (row #20) remains to be better defined and investigated before measurement approaches are sought.
2. Physicochemical sensors for spatially distributed measurements of fields of variables (*e.g.*, maps of analyte concentrations) over a variety of timescales shorter than those of space missions (years or less). There exist sensors able to measure a broad array of physicochemical indicators (*e.g.*, those of rows #7 and 17 in Table 4). However, technology is lacking for their routine, repeated, short-turnaround distributed use as an agnostic means of searching for catalytic activity overprinted on an abiotic background (*e.g.*, rows #8–9 and 19).
3. Instruments for detecting and characterizing a variety of untargeted informational heteropolymers. Nanopore sequencing technology and mass spectrometry allow identification of primary structure of DNA and oligomers (Mojarro *et al*., 2019; Špaček and Benner, 2022). The former remains Earth-centric and with low technical readiness level, although ongoing efforts are addressing both challenges.

   Technology development is also ongoing for protein sequencing (Reed *et al*., 2022) and for directly measuring higher level protein structure (*e.g.*, by electrochemical atomic force microscopy).

Unlike for measurement technologies, there are currently broad gaps regarding sample handling (Section 3.2) that are being addressed only by a handful of instances of incipient technology development. Gaps identified during the workshop include the following:

4. Technology to process liquid and frozen samples. Search-for-life measurements routinely require liquid samples (Table 4), at least during sample processing steps. Yet it is challenging to process (move, filter, degas, label, mix, concentrate, etc.) liquid at the low temperatures and/or low pressures typical of sampling locations in planetary environments targeted in the search for life. The properties of the samples of interest often add challenges: samples may be diluted, their amount may be limited, and contamination must be mitigated typically to levels as low as or below analyte concentrations.
5. Technology and associated sampling strategies to preserve the thermal, mechanical, and chemical integrity of the samples upon acquisition. Specific needs to preserve these properties depend on the potential biosignature sought and pertain especially to solid samples, although these considerations can also be relevant to liquid samples.
6. Technology and associated sample handling strategies to optimize sample information content based on sampling location and sample consumption. This requires the ability for instruments to work together in suites. The needed level of coupling and integration far exceeds that of current state-of-the-art spaceflight investigations, presenting technical, organizational, and operational challenges. A drawback associated with the tools and mindset that have historically been used in mission concept development and evaluation is that they lend themselves to distinct instrument development and operation, with self-contained instrument teams, proposals or proposal sections, and

development schedules. Crucially, search-for-life investigations, in contrast, will require integration from concept inception, to development, to operation to reconnoiter sampling locales at nested spatial scales and perform sequences of increasingly destructive analyses on shared samples.

5. Workshop Outcomes and Lessons Learned

To date, lander-based *in situ* measurement technologies used for solar system exploration have been limited relative to the vast scope of technologies available for pharmaceutical, medical, forensic, environmental, industrial, and other fields that are used to examine the manifestations of life on Earth. Considered in this light, a primary goal established for the FoSL workshop was to facilitate a community discussion, not on what has been done or has already been put forth in mission studies, but on what might be possible for future search-for-life missions. To achieve broad and diverse perspectives, the workshop announcement stated that the organizers ''especially seek participation and encourage applications from engineers and scientists outside of the traditional life-detection community.'' The workshop application, in addition to requesting information on participant career stage, provided potential participants the opportunity to describe science and/or engineering experiences and interests that they believed might be relevant to this workshop.

These responses were carefully considered when selecting the 100 workshop participants from more than 350 workshop applications. The selection of applicants who had prior experience with planetary mission science formulation and requirement development was intentionally limited and reflected by 65% of participants falling into the categories of early career, postdoc, and graduate student. In addition, a 30% cap was placed on NASA-center scientists and engineers (both contractor and civil servant) and 35% cap on mid-/senior career stages. Accordingly, many of the selected participants did not have prior experience with science mission development.

The intent of bringing to the table dozens of people with extensive expertise in their field, but without direct science-mission planning and implementation experience, was to help foster new ideas. In hindsight, use of the NASA STM as a tool to formulate and record these ideas presented challenges. An STM is a required component of NASA science mission proposals, used to distill a mission concept from science objectives and requirements down to instrument and mission requirements. It provides a tabular summary of objectives and requirements that are fully described in the proposal.

Extensive expertise and a tremendous team effort, typically over several months, are required to formulate a well-crafted mission concept and to structure it into a robust STM. This was not a possible outcome of the ~40-h FoSL workshop. Instead, the STM served as a framework for thought and discussion with the intent to connect science ideas with measurement needs over the course of the two-part workshop.

The workshop's discussions fully achieved its goal of connecting scientists and engineers, as well as people previously engaged in planetary science missions with those outside that community. These discussions resulted in an outpour of ideas for search-for-life measurements and sample handling. Some ideas were new, others previously considered for search-for-life missions. Many ideas straddled these end-members as updates from existing ideas, transposed to the new environmental contexts expected to become within reach of robotic spacecraft in the 2040s and beyond. This raised the challenge of distilling and harmonizing ideas of heterogeneous detail and maturity, emphasizing more novel and thus lesser understood measurements and environmental contexts, into the STM format designed to convey rationalized, quantified, and actionable requirements.

Consequently, the measurements and sample handling needs detailed in Table 4 reflect ideas that need further exploration to reach the level of realism and actionability needed to initiate the development of instrument or sample handling hardware able to address these needs (Section 4). The connections initiated during the workshop, and this report to the broader community, form the seed of this follow-on work. These lessons learned from the FoSL exercise suggest that further idea development could be better addressed by one or several smaller groups working asynchronously over the course of several months punctuated by short meetings, for example, akin to Science Definition Teams.

6. Concluding Remarks

The overarching goal of the FoSL workshop was to bring scientists and engineers together to collectively develop new and creative approaches to *in situ* searches for life elsewhere in our solar system, 20 years or more in the future. The backgrounds of the workshop participants addressed the workshop goals; the proportions of 2/3 scientists to 1/3 engineers reflected an even larger proportion of scientists among applicants. Logistical constraints capped the participant count at 100, preventing active participation of three to four times as many interested people, who were, nevertheless, able to watch the plenary presentations. As such, the outcomes of this workshop do not encompass the viewpoints and ideas of everyone seeking to participate in this exercise and reflect mainly those of participants from institutions in the United States.

Emphasis was placed on three indicators of life identified by the workshop scientific organizing committee as particularly understudied to date (Section 2.2), planetary environments that are largely beyond the reach of current spaceflight capabilities (Section 3.1), and sample handling considerations that have not been emphasized in past mission concept solicitation documents (Section 3.2). Notably, understudied indicators formed only a minor part of the pool of indicators of life defined by the participants (Fig. 3). Ongoing technology development is seeking to make *in situ* sampling of environments considered habitable today a concrete prospect for 20 years from now. The expanding portfolio of environments amenable to *in situ* sampling and of measurements requiring sample preparation is likely to require a comparably growing emphasis on sample handling and its integration within instrument suites in the next two decades to usher in the future of the search for life and its characterization, if located.

Acknowledgments

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The workshop was advertised through avenues such as the Planetary Exploration Newsletter; distribution lists of the NASA Science Mission Directorate, Lunar & Planetary Institute, IEEE Aerospace and Electronics System Society, NASA Astrobiology Program, NASA Network for Life Detection, NASA Network for Ocean Worlds, and American Astronomical Society's Division for Planetary Sciences; the NASA Astrobiology Program and IEEE Aerospace and Electronics System Society websites; and the Facebook group Early Career Planetary Explorers.

Authors' Contributions

M.N. and R.Q. co-chaired the FoSL workshop. They and L.M.B., K.L.C., C.R.G., S.A.G., C.G., and M.P. comprised the workshop's Scientific Organizing Committee. The other coauthors (Workshop Report Contributors) organized the notes, science traceability entries, and sample handling flowcharts of their respective breakout groups, which were further edited by M.N. for harmonization. M.N. wrote a first draft of the article with text and figure contributions by R.Q. Scientific Organizing Committee authors edited the first draft. All authors edited or otherwise provided feedback on the article.

Author Disclosure Statement

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Supplementary Material

Supplementary Data

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Abbreviations Used

FoSL = Future of the Search for Life
LDF = Life Detection Forum
LDKB = Life Detection Knowledge Base
LoD = limit of detection
STM = Science Traceability Matrix
U = university