Developing a Fundamental Understanding of Workplace Backgrounds:

Organic and Organismal Basics for Life Evaluation and Contamination Knowledge

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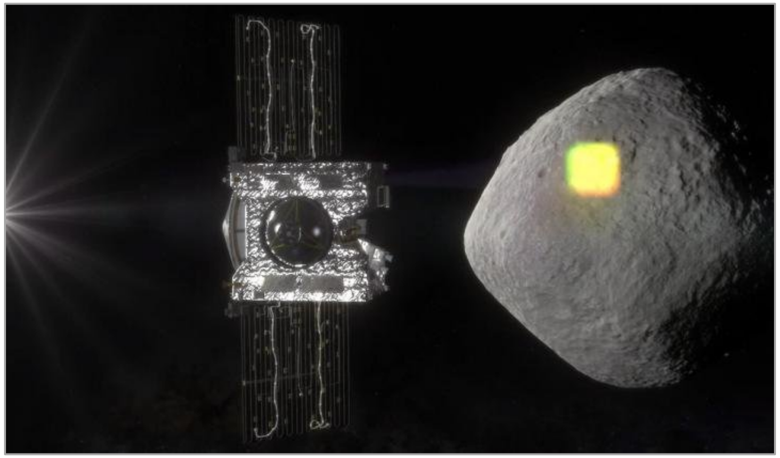
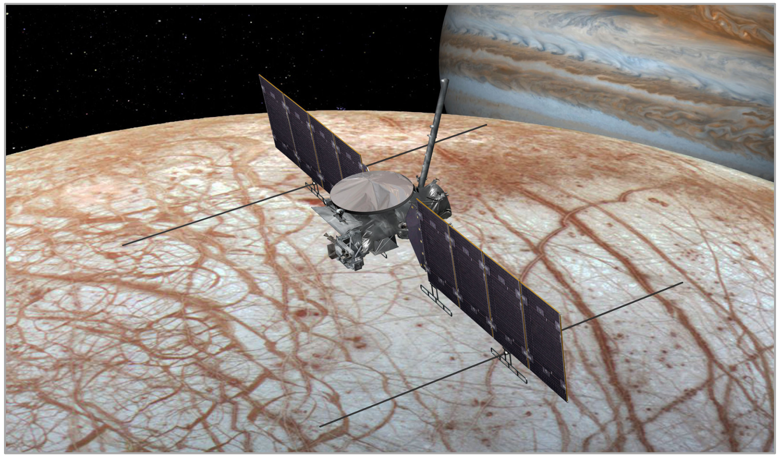
Planetary Protection for Astrobiology

- Prebiotic Chemistry
- Life Detection
- Sample Return Missions

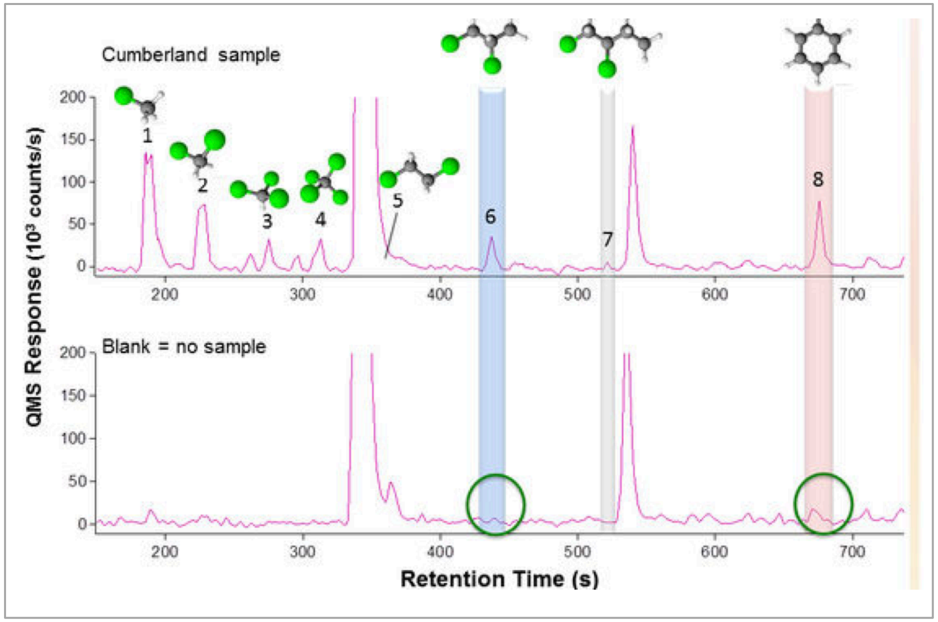




Planetary Protection for Astrobiology

The identification of well-established and widely accepted organic molecules associated with terrestrial life and signatures of biologic processes.

- **Particular classes, patterns, and isotopic signatures of organic molecules by mass spectrometry.**



Planetary Protection for Astrobiology

For many astrobiology planetary exploration missions the scientific cleanliness requirements often exceed Planetary Protection bioburden requirements.

Contaminant Class	Examples
Nucleic acids	DNA
Spores	Dipicolinic acid
Bacterial and fungal cell walls	N-Acetylglucosamine
Amino acids	Glycine, Alanine
Lipids	Palmitic acid, Squalene
Hydrocarbon biomarkers	Pristane
Martian organics	Chlorobenzene, Dichloromethane
PAHs	Naphthalene
Nitrogenous compounds	Urea
Short-chain carboxylic acids	Acetic acid
Polyhydroxy compounds	Glycerol
Hydroxy carboxylic acid	Pyruvic acid
Linear hydrocarbons	n-Heptacosane

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Sample	Total amino acids
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Residue from Kimtech glove on foil	0.97 ng/cm^2
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Chlorobenzene abundance on Mars = ~200ppm
Total amino acid abundance in meteorites = 44 to 6300 ng/g

Planetary Protection for Astrobiology

Operational definition of "clean" for astrobiology.

CLEAN **means that no foreign material is introduced to the sample in an amount that hampers the ability to analyze the chemistry of the sample.**

Sample Species	Allowable abundance ng/g
Aromatic hydrocarbons	8
Carbonyls & hydroxyls	10
Amino acids	1
Amines or amides	2
Aliphatic hydrocarbons	8
DNA	1
Total reduced carbon	40

Planetary Protection for Astrobiology

Biosignature and life detection missions require careful attention to restricting terrestrial organic contamination that can be easily convoluted with analytical targets

- ***KNOWLEDGE NEEDS for astrobiology and sample return missions***
- ***METHODS for building an organic contamination knowledge dataset***
- ***APPLICATIONS for knowledge products***

Selected PPIRB Findings

- *For planetary missions to locations of high astrobiological potential it is essential that forward and backward contamination consideration be integral to mission implementation.*

- *Because of advances in knowledge and technologies since the Viking era, Planetary Protection policies and procedures should be reassessed.*

- *The PPIRB encourages flexible ways to address the intent of Planetary Protection using novel methods and the use of modern molecular biological approaches for analyses of cleanroom samples.*

Contaminant Knowledge for Astrobiology

1. A general organic contamination knowledge dataset for sample curation facilities and spacecraft assemblies
2. An understanding of the effects of bioburden reduction protocols on organic contamination loads
3. Novel methods to discern contribution from bioburden to organic background

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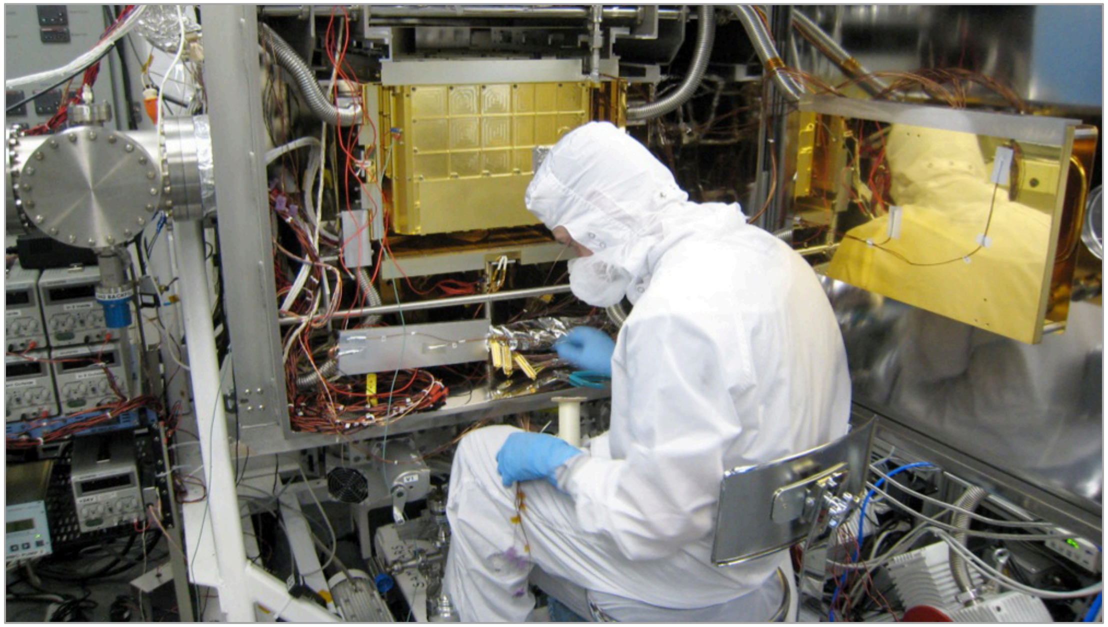
  - We recommend an ongoing organic reconnaissance cataloging small molecules, biomolecules, volatiles, and hydrocarbons that identifies and quantifies contaminants by mass spectrometry.

Organic Contaminant Knowledge Census

- *The ability to deconvolve false positives is relies on a thorough understanding of the identity and behavior of potential contaminants*



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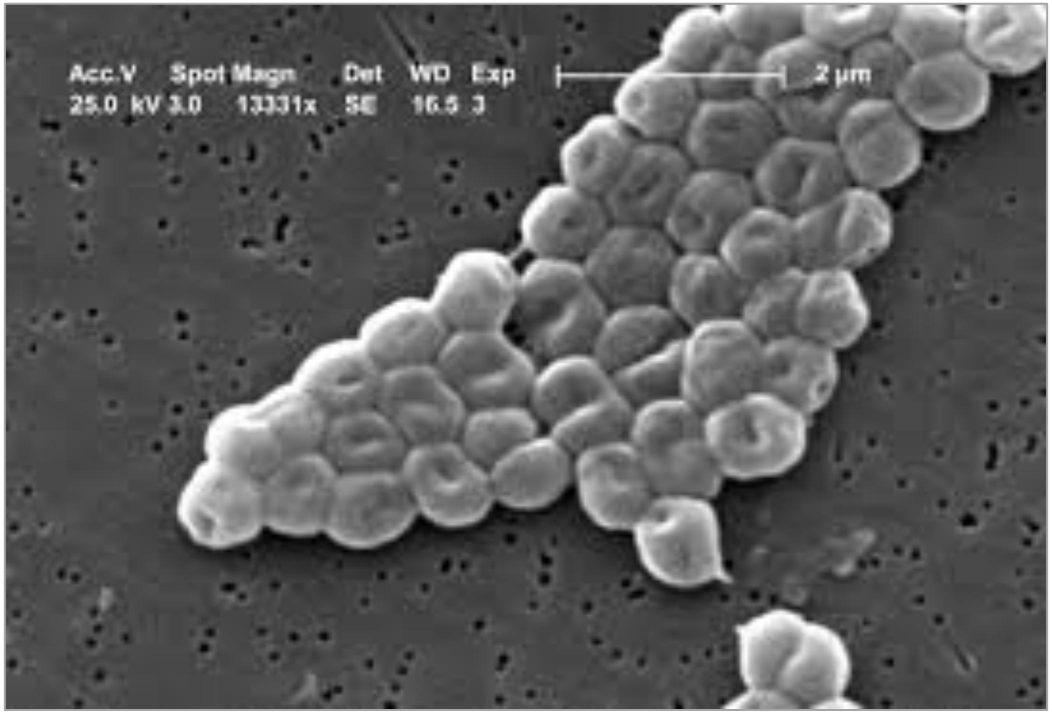
  - We recommend compound-specific organic characterization of surfaces cleaned for microbe reduction

Evaluating Effects of Bioburden Reduction

- *Heat and solvent cleaning incompletely remove organics and necromass, leaving behind potential target compounds and might even select for certain resistant species*



Connecting Bioburden with Contaminants

Novel methods to discern contribution from bioburden to organic background

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  - Current bioburden assays defined by spore surface density and ATP abundance
  - Assays are designed to assess resistant bioburden rather than quantifiable viable or residual organisms
  - We recommend new methods that allow for the identification and quantification of residual organisms, living or dead, at a very broad level for any bacteria

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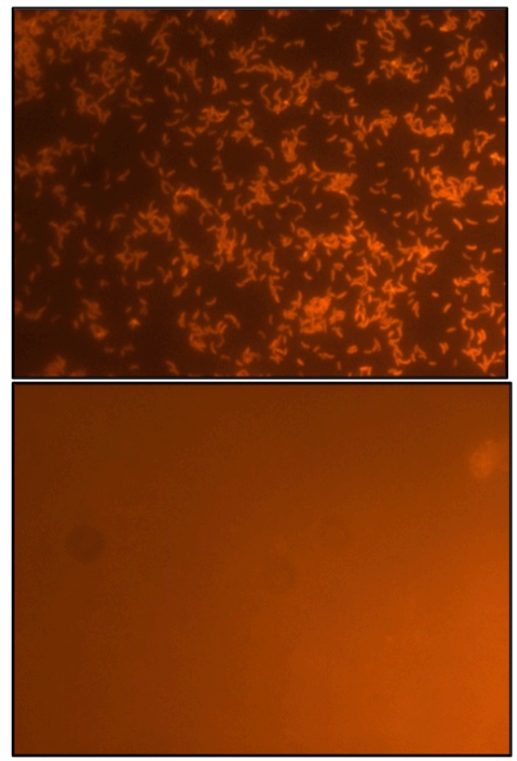
– *"novel methods and the use of modern molecular biological approaches for analyses or cleanroom samples"*

- **FISH (fluorescence in situ hybridization) is a preferred method for low biomass accumulations**
- **Allows quantification**
- **Probes can be tailored for type to give phylogenetic and biodiversity data**



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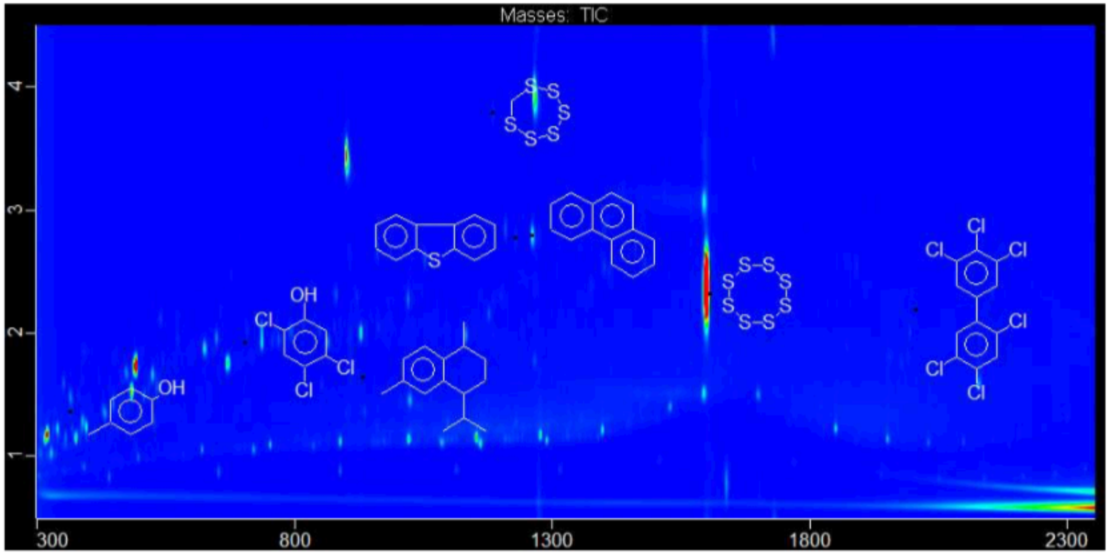
- **Two-dimensional GCMS (GCxGCMS) for better sourcing of mixed sources or altered species. Can deconvolve co-eluting peaks and identify indeterminate functional groups.**



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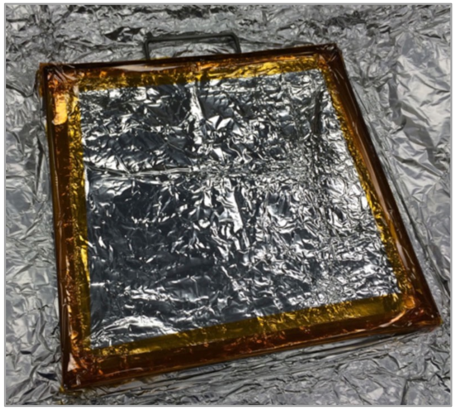
- **In addition to witness foils and wafers SPME (Solid Phase Micro-Extraction) fibers can be used to collect a variety of airborne organic compounds. SPME fibers can be optimized for analyte collection by polarity and volatility.**



Organic Contaminant Knowledge Uses

An ongoing organic contamination knowledge record for small molecules, biomolecules, volatiles, and hydrocarbons in assembly and curation facilities would help:

- *Constrain inputs for the Europa Probabilistic Model of Planetary Protection minimums and bioburden fate*
  - *Model lacked complete knowledge of initial organic contaminant load*
- *Update baseline characterization data for curation facilities*
  - *With the imminent arrival of Bennu and Hayabusa2 samples it is necessary to qualify the facility currently under construction*
- *Allow comparative evaluation of the cost-effectiveness of differing planetary protection protocols*
  - *OSIRIS-REx levels of pristine vs other missions*

OOBLECK

- ***Thanks to my Organic Contamination Knowledge colleagues***

Jason Dworkin/GSFC, OSIRIS-REx Project Scientist
Aaron Regberg/JSC, Astromaterials Research and Exploration Science Curator
Erin Lalime/GSFC, Europa Clipper Planetary Protection Engineer
Melissa FLoyd/GSFC, Research Microbiologist

. Please see recent PPIRB at

https://www.nasa.gov/sites/default/files/atoms/files/planetary_protection_board_report_20191018.pdf