National Aeronautics and Space Administration

SCHAN: Analysis of biomolecules from resilient microorganisms using supercritical CO_2 and subcritical H_2O

Principal Investigator: Bryana Henderson (322); Co-Investigators: Victor Abrahamsson (382), Fang Zhong (382), Ying Lin (411), Wayne Schubert (353), Fei Chen (353), Michael Tuite (322), Jens Prothmann (382), Amy Williams (University of Florida)

Program: FY22 R&TD Strategic Initiative
Strategic Focus Area: In-Situ Extant Life Detection Technology - Strategic Initiative Leader: Victor Abrahamsson

Objectives:

1. Demonstrate the use of supercritical CO_2 for lysing microbes in order to analyze organic biosignatures, inside microbial cells suspended in liquid samples, at parts-per-trillion (ppt) concentration levels and ≤10^5 cells/mL.
2. Demonstrate that carbonated water (pH of approx. 3-4) can hydrolyze proteins into amino acids and small peptides.
3. Couple SCHAN with an ice/liquid handling and delivery subsystem and demonstrate an end-to-end analysis.

Background:

Life-detection missions to Enceladus and Mars have been identified as two of the top priorities for the upcoming decade by the Decadal Strategy for Planetary Science and Astrobiology 2023-2032. New technologies, and sensitive and versatile instruments such as SCHAN are needed to fulfill stringent mission concept measurement requirements. SCHAN offers several advantages over its competitors in terms of sensitivity, simplicity, and gentleness of approach because no harsh chemicals or extreme temperatures are used.

The SCHAN instrument is fully capable of analyzing both solid samples (Mars) and liquid samples (melted ice from Enceladus). This task has so far focused mainly on an Enceladus scenario. During FY22, SCHAN has developed additional capabilities for detecting extant life by lysing, accessing, and analyzing organic biosignatures inside of microbes.

Approach and Results:

The operational steps of microbe analysis with the SCHAN instrument are as follows: 1) load sample and capture microbes on a filter, 2) perform cell lysis with supercritical CO2 (22 MPa, ≤250 °C), 3) extract, preconcentrate, separate, and detect organic biosignatures (Fig. 1).

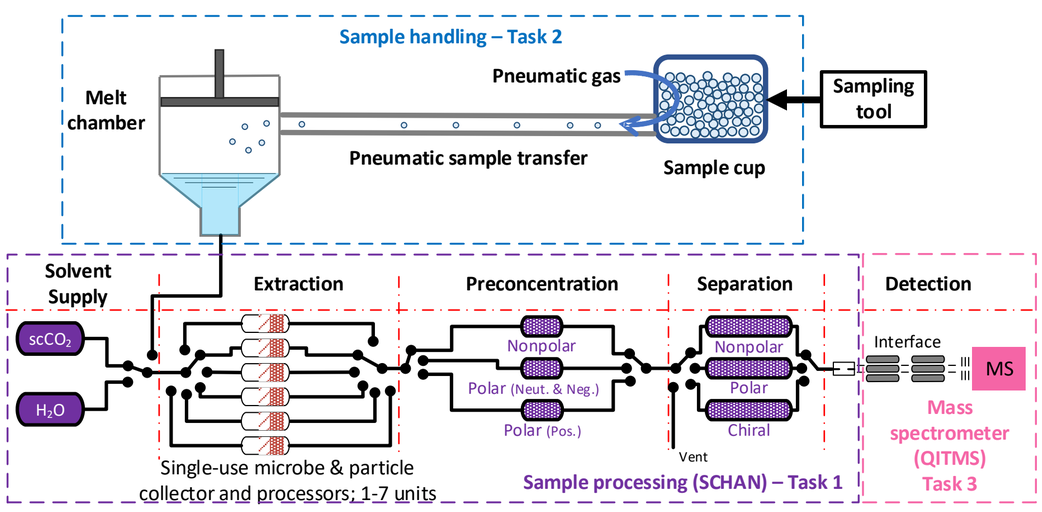

Figure 1. Schematic overview of the overall Initiative. The SCHAN instrument was tested and validated as a stand-alone instrument. Extracted organic biosignatures were subsequently preconcentrated, separated (chromatography), and detected, with extremely low LODs.

National Aeronautics and Space Administration

Jet Propulsion Laboratory
California Institute of Technology
Pasadena, California

www.nasa.gov

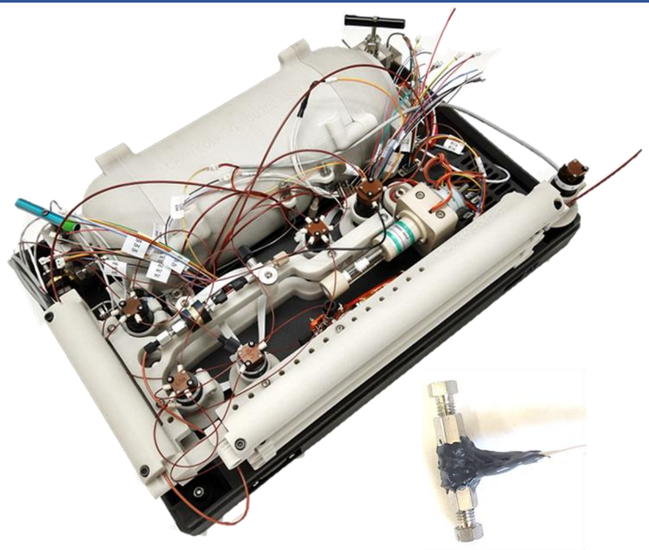

Figure 2. The brassboard SCHAN instrument that was used for the analog sample validation with seawater. The filter holder equipped with a heater and temperature sensor is shown on the right (not to scale).

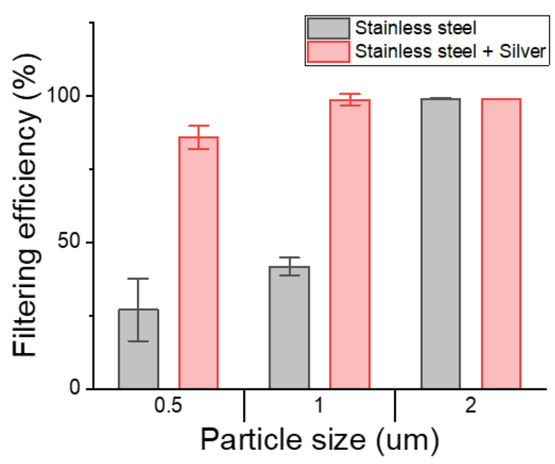

Figure 3. Filtering efficiencies were evaluated by passing through fluorescent microbeads that were later quantified with a spectrofluorometer. The stainless-steel filters are sufficient for viable microbes (also confirmed with *B. atrophaeus*, 99% filtering efficiency). The addition of thin silver membranes could be used to successfully capture spores so that their contents can be lysed and analyzed.

Step 1 (filtering) was optimized by examining the filtering efficiency of various porous metal filters using fluorescent microbeads.

Step 2 (lysing) was optimized using *B. atrophaeus* (a gram-positive bacteria with a very study cell wall) and confirmed with other microbes. Lysis was performed using supercritical CO_2 with temperatures ranging from 50 – 250 °C and over 15 to 90 minutes. The highest signal, which provides the lowest LOD, was found at 250 °C and 30 minutes.

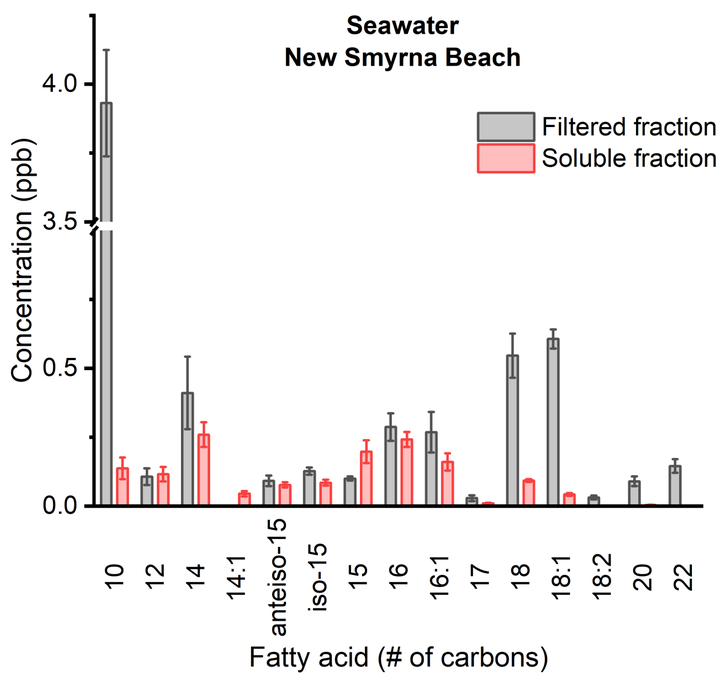

Figure 5. The SCHAN brassboard analyzed an Ocean World analog sample from the Atlantic Ocean using the methodology developed during FY22. Many fatty acids were found and quantified, most at ppt levels. No other instrument is capable of detecting fatty acids at ppt level, showing that SCHAN is uniquely capable of detecting extant life at these low concentrations.

The brassboard SCHAN instrument was able to detect 1×10^4 cells/mL of *B. Atrophaeus* with the optimized method. An analog sample (seawater from the Atlantic Ocean) was analyzed with SCHAN (Fig. 4). Additionally, hydrolysis of proteins into amino acids and peptides with carbonated subcritical water (pH approx. 3 to 4) was demonstrated. Finally, the sample handling and distribution subsystem was integrated and verified together with SCHAN.

Significance/Benefits to JPL and NASA:

The SCHAN is the most sensitive instrument in the world for in-situ lipid biosignature analysis. SCHAN is also unique in its capability of completely integrated sample processing for microbe lysis coupled with chemical analysis without organic solvents, and is capable of detecting 1×10^4 cells/mL.

Publications:
[1] Henderson, et al., 2022 Astrobiology Science Conference, AGU, Atlanta, GA, 2022.
[2] Abrahamsson, et al., 2022 Astrobiology Science Conference, AGU, Atlanta, GA, 2022.

PI/Task Mgr. Contact Information:
Email: Bryana.L.Henderson@jpl.nasa.gov