**Surmounting Return Sample Science Barriers with NRL's NAUTILUS: The Next
Generation of Secondary Ion Mass Spectrometry**

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INTRODUCTION

The search for ancient life on Earth is a complicated task fraught with numerous challenges and uncertainties. The most limiting aspect is the fossil record, which becomes increasingly scarce the further back in time you go. Any purported fossils that are discovered are often controversial, owing largely to the fact that this early life preserved in the fossil record is entirely comprised of microbial fossils (microfossils) which are simple in morphology, can be mimicked by non-biological phenomena, and often are not well preserved.

Now, led in large part by NASA's Mars 2020 mission, the search for life outside of our planet is an active research area with many challenges for the scientific community at large. The burden of proof for confirming the existence of life outside of our planet will be unprecedented in scientific history, with positive results bearing immense implications for the commonality of life in the universe and the ability to find it elsewhere. Such a discovery would undoubtedly spur a paradigm shift to the Earth and planetary sciences, as well as shape mission and funding priorities on a global scale for the foreseeable future. Leading in the efforts to characterize past and present habitable environments and search for evidence of life outside of Earth are rovers such as NASA's *Curiosity* and *Perseverance*, which are equipped with powerful analytical instruments that can provide useful context data, but are typically of lower resolution than similar laboratory-based counterparts due to payload constraints. Return Sample Science (RSS) missions allow for the use of the highest precision laboratory-based instruments, including secondary ion mass spectrometry (SIMS), which allows for unique and in-depth sample analyses that would otherwise not be possible from a solely rover-based mission. This is especially true for the *Perseverance* mission, which seeks to identify signs of ancient life, which necessitates analyses on a molecular to micron scale, via the search for biomarkers, microfossils, etc.

The ability to verifiably ascribe a biological origin to suspect fossils is entirely contingent on the successful application of robust biosignatures, leading to high confidence results consistent with a biological interpretation. Developing robust biosignatures in ground-based laboratories is critically important, supporting *Perseverance*'s primary mission objective of searching for evidence of past life as it traverses Jezero crater. These robust biosignatures will be the foundation upon which the biogenicity (biological origin) of potential microfossils in future Martian return samples will be ascertained. Finding microfossils would provide the most direct evidence of life, but even on Earth the biogenicity of ancient microfossils is often debated owing to simple morphology and highly altered host rocks¹⁻³. Since morphology alone is often not sufficient for proving biogenicity, positive identification of biogenicity requires multiple lines of supporting evidence. Several biosignatures exist, but some analyses require complete sample consumption (e.g., combustion for elemental analysis isotope ratio mass spectrometry) and therefore can be mutually exclusive. This factor will be particularly relevant for samples collected by the *Perseverance* rover and later returned to Earth for the RSS component of the Mars 2020 mission. The small sample size, limited sample number, planned storage of some portion of all returned samples in a curation facility for future scientific efforts, and extremely high demand of any remaining samples deemed fit for laboratory-based analyses will undoubtedly merit extremely strict analytical methods to be employed in order to ensure that any of these precious samples are preserved to the highest possible extent.

Here we describe the Naval Ultra Trace Isotope Laboratory's Universal Spectrometer (NAUTILUS), a new, globally unique instrument, which is well suited to obtain minimally

destructive yet highly informative data in samples that will be collected and returned to Earth by the Mars 2020 mission and other future RSS missions. The NAUTILUS employs the minimally destructive and highly informative capabilities typical of SIMS and other in situ mass spectrometer techniques, while allowing for direct, uncorrected, high-accuracy, and high-precision measurements to be obtained even for complex samples. For these reasons, we propose the NAUTILUS as a top contender for future RSS analyses.

INSTRUMENTAL BACKGROUND AND PRIOR LIMITATIONS:

The NAUTILUS at the U.S. Naval Research Laboratory combines two mainstay analytical techniques, SIMS and accelerator mass spectrometry (AMS), into one unified instrument (described completely in Groopman et al. 2020 ⁴). This combination of instruments is complementary, and when combined, mitigates many of the drawbacks associated with each technique when applied separately. SIMS is known to be the highest-sensitivity surface analysis technique, typically achieving lateral and depth resolutions on the scale of micro- and nanometers, respectively, and trace element sensitivities down to nmol·mol levels. Recently, SIMS has been suggested as a promising analytical technique for analyzing modern and ancient microbial remains in Mars analogue environments, using elemental sequestrations as unique chemical biosignatures ^{5,6}. AMS is used for ultra-trace sample analysis with the highest level of specificity (e.g., the ability to resolve analytical interferences). Each technique excels at certain kinds of analyses. SIMS is typically used to target micrometer-scale mineral phases in situ, and to resolve ion-implantation depth profiles in semiconductors, while AMS is typically used to measure ultra-trace abundances of radioisotopes, such as ²⁶Al and ⁶⁰Fe at concentrations down to 10⁻¹⁵. However, a capability gap remains between these two (and other) mass spectrometry techniques, which the NAUTILUS addresses. This capability gap is highly important to the development of robust biosignatures: namely, the ability to spatially resolve (image) minor and trace element abundances with high specificity.

The NAUTILUS (Figure 1) can best be thought of as a SIMS instrument with an additional molecule-filtering AMS “detector”. Sample preparation and analysis is SIMS-like, however the AMS component mitigates the presence of molecular isobars (molecules with the same mass as elements of interest) in the mass spectrum, which are one of the greatest drawbacks of SIMS and are especially problematic for trace element and high-mass (> 50 amu) analyses. During the sputtering process, both atomic and molecular ions are produced. From complex (e.g., geological) samples, the molecular ion spectrum can confound precise analyses, and can even be orders of magnitude more abundant than high-mass elements of interest (e.g., Groopman et al. 2017 ⁷) making analysis impossible. The NAUTILUS removes molecular isobars with the AMS component and achieves instrumental background levels of <1 count/hour, enabling ppb–100ppt measurements (depending on atom limitations), which is far below the detection limits of conventional SIMS instruments ⁴. Conventional SIMS instruments typically employ increased mass resolving power (MRP; $M/\Delta M$: usually defined as the full peak width at 10% peak height) to distinguish true elemental/isotopic signals from molecular isobars. However, increasing the MRP decreases instrumental transmission, precision, and instrument stability. Additionally, some molecular isobars require impractically high MRP to resolve, such that instrument transmission would be essentially zero (e.g., $M/\Delta M = 17,000$ to resolve ⁸¹Br & ⁵⁴Fe²⁷Al). Traditional, standalone AMS resolves nuclear and molecular isobars by accelerating ion beams

Figure 1: 3D CAD model of the NAUTILUS with SIMION cutaway view. The NAUTILUS is a standalone SIMS instrument with a single-stage accelerator mass spectrometer “detector” that eliminates molecular isobaric interferences. Figure and caption taken from Groopman et al., 2020 ⁴.

to very high energies and using energy-sensitive detectors to distinguish small differences in mass. However, milligram-to-gram-sized samples are typically required by AMS in order to achieve a dynamic range up to 10^{15} , and these samples are often first dissolved and concentrated using chemistry. This can be detrimental to robust biosignature work, since important information such as morphology and geologic context is lost.

Specifically, the NAUTILUS uses a single-stage AMS (SSAMS), which instead of being limited to negative ions like the more common tandem AMS, can also measure positive ions. The result is greatly increased electropositive elemental sensitivity (i.e., most trace/high-mass elements) relative to tandem AMS, which must inject electropositive elements as negative molecular ions. Positive ion yields are orders of magnitude higher than negative molecular ions when analyzed on the SSAMS component. This increased sensitivity to electropositive elements makes it possible to measure elements on a micro scale, allowing for accurate quantification as well as imaging capabilities vital for correlating elemental distributions with morphology (especially relevant here for microfossil-related research).

In contrast, the detection limits for conventional SIMS instruments are commonly variable and highly sample dependent (i.e., samples needing to have a non-heterogenous/low background), and factors such as molecular background may lead to misinformed analyses that can cause complete misinterpretation of sample data (e.g., Figure 2). The NAUTILUS’ sensitivity to trace elements is at least $10\times$ better than commercial SIMS instruments due to the near-zero background conditions of the instrument ⁴. Typically, conventional SIMS employ O^- beams for trace element analyses, which can be effective, but is limited due to the small beam size and poor depth resolution, making it sub-optimal for many analyses. Utilizing

Figure 2. Europium isotope ratio imaging of Oklo nuclear fuel on the NAUTILUS, highlighting molecule-free imaging capabilities of the SSAMS (upper panels) and SIMS images (lower panels). Europium-151 is more highly depleted than Europium-153, but this data is obscured by a large, complex molecular background on the SIMS. The SIMS images also show several false-positive features, and the isotope ratio image (right) is almost an exact inverse of the true ratio image. Figure taken from Groopman et al., 2018 ⁸.

molecular/fluorinated ion beams and flooding gases can greatly increase sensitivity to many trace elements, but also lead to a much greater and more varied molecular background on conventional SIMS. The AMS component of the NAUTILUS removes these issues, making novel beams and flooding gases an additional useful tool that can greatly enhance analytical capabilities. Preliminary tests have resulted in the successful production of several fluorinated and molecular ion beams (e.g., CFO^- , NO_2^- , NO_2^+), which can greatly enhance the secondary yield/sample counts of certain elements ($10\text{-}100\times \text{O}^-$), especially when combined with various flooding gases ⁴. These new measurement paradigms introduced by the NAUTILUS provide multiple benefits such as reduced background, increased sensitivity, and the ability to tailor primary beam species based on sample type and targeted data of interest. Such capabilities will benefit the development and detection of robust biosignatures, surmounting prior technological barriers and allowing for the successful acquisition of vital sample data which may otherwise have been unobtainable due to prior instrumental barriers (e.g., detection limits and sample complexity).

RSS APPLICATION POTENTIAL:

Due to the unmatched analytical capabilities of the NAUTILUS, it is well suited for a variety of RSS applications. The most pressing RSS effort currently at hand is that of the Mars 2020 mission, which has an RSS component specifically targeting analyses which are otherwise unobtainable by rover measurements or other common laboratory instrumentation ⁹. A top priority for the astrobiology science community during the next decade is to have high-impact

Figure 3. SIMS mapping of ancient life. Left: Confocal Laser Scanning Microscope image of a ~350 million-year-old microfossil. The white box indicates the area targeted for SIMS analyses (where the microfossil bisects the surface of the sample, and is thus able to be analyzed), with subsequent panels showing the SIMS-generated elemental maps for the noted element. Color bar shows relative intensity from low (black) to high (red). Figure adapted from Gangidine, 2020 ⁵.

RSS instrumentation/techniques at the ready awaiting the return of the *Perseverance* rover sample haul. As a primary mission objective of the Mars 2020 mission is to search for and characterize evidence of past life ¹⁰, biosignature-related analytical techniques are especially important. A new biosignature based on SIMS measurements of trace element sequestrations in microbial remains in Mars analogue environments has recently been described ^{5,6}, but thus far this technique has only employed conventional SIMS instrumentation and a fraction of elements on the periodic table. SIMS has shown that trace elements appear to be preferentially retained in microbial remains and microfossils in hot spring deposits dating as far back as the mid-Paleozoic (Figure 3)⁶. While this method proved useful for a variety of elements, detection limits prevented the successful quantification of other elements due to low counts being obtained. This issue was caused by the complex molecular background in the samples analyzed and detection limits of the SIMS instrumentation used ^{5,6}. The NAUTILUS would allow for far more robust measurements using a similar technique, which has already shown promise using conventional SIMS instrumentation. Future analyses of these samples using the NAUTILUS should allow for the successful characterization of lower-abundance elements. SIMS has already been employed on samples from terrestrial (land-based) hot springs, settings on Earth which are known to well-preserve evidence of life, and are also thought to exist on Mars ^{11,12}, perhaps even on the crater rim of Jezero ^{13,14}, which is a planned sampling site of the *Perseverance* rover. As the most optimistic date for the arrival of samples from the Mars 2020 mission is 2029, this technique can be further fleshed out and developed in the next several years. This development will be guided by the findings of the *Perseverance* rover, with in situ rover measurements informing a broad scale picture of sample composition and primary elements of interest in samples drilled and cached by the rover for future analyses. These unprecedented questions sought to be answered by the Mars 2020 mission require substantial scientific advancements. The globally unique instrument strengths of the NAUTILUS will yield not only a high degree of sample preservation in any analyzed samples but also highly informative data on RSS samples from the Mars 2020 mission and beyond.

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