# Mars Technology Program Planetary Protection Technology Development

Ying Lin
Jet Propulsion Laboratory, California Institute of Technology
4800 Oak Grove Drive
Pasadena, CA 91109
818-393-6381
ying.lin@jpl.nasa.gov

*Abstract*—The objectives of the NASA Planetary Protection program are to preserve biological and organic conditions of solar-system bodies for future scientific exploration and to protect the Earth from potential hazardous extraterrestrial contamination. As the exploration of solar system continues, NASA remains committed to the implementation of planetary protection policy and regulations. To fulfill this commitment, the Mars Technology Program (MTP) has invested in a portfolio of tasks for developing necessary technologies to meet planetary protection requirements for the next decade missions.[1,2]

The MTP planetary protection technology development program comprises seven tasks:

1. Cleaning to achieve sterility
2. Rapid single spore enumeration assay
3. Light weight biobarrier technology
4. Spore adhesion for contamination transport model
5. Near field and integrated particle transport model
6. Mars orbital debris analysis tool
7. Contained sample handling and analysis

In this paper, we will give an overview of the task objectives, current technical progress, and future directions.

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## 1. INTRODUCTION

*Background*

During the preparation period of the first extraterrestrial biological exploration mission known as the Viking Project in 1950's, the international community, represented by the Committee on Space Research (COSPAR) of the International Council of Scientific Unions adopted a set of recommendations for the protection of planets and for the reduction of viable microorganisms on spacecraft [1, 2]. In 1967, UN Space Treaty referred to as Article IX of the *Treaty on Principle Governing the Activities of States in the Exploration and Use of Outer Space Including the Moon and Other Celestial Bodies* was accepted by the United States [3]. In 1968, COSPAR convened its first symposium and published a set of quantitative guidelines (8020.12/12A) for implementing planetary protection (PP). In the guidelines it defines planetary protection as "*the avoidance of contaminating the biosphere of a planet with terrestrial life forms so that the ecology of a planet is maintained in its pristine state during the period of scientific investigation*"[4]. In the same year, NASA established a planetary protection office to put these guidelines into effect.

In the past 40 years, NASA has focused its extraterrestrial life searching activities on Mars. A series of planetary missions to Mars were completed and numerous scientific findings resulting from those missions have increased our understanding of the Martian environments and its potential to harbor biological life. As a result, the development and implementation of the planetary protection policies are continuously evolving. A series of significant changes of the planetary protection policy have been published over the last 25 years [5-14]. The PP implementation methods used by Viking mission have been replaced by current implementation policy based on five categories of planetary missions and the PP requirements defined by each category.

*The NASA Planetary Protection Policy Mission Categories and Planetary Protection Requirements*

Each planetary mission falls into one of five categories. Category I missions are aimed at targets of no direct exobiological interest e.g. the sun and the moon. There is no planetary protection requirement imposed on such missions. Category II missions are missions to planets which have significant interest in the process of chemical evolution but only a remote chance that contamination from

spacecraft would compromise future exploration. There is no formal implementation requirement for category II mission either. Category III missions are for flyby and orbiter missions aimed at a planet of significant interest relative to the process of chemical evolution and the origin of life. There exist significant chance that contamination from spacecraft will jeopardize a future biological experiment. Category IV missions are Lander and Probe type missions aimed at the planet similar to those in category III missions. In 1992, COSPAR established two separate sub-categories with Category IV missions. Category IVa are missions utilizing landers and probes but do not carry life detection experiments. It requires bioburden reduction of spacecraft but not full sterilization as Viking required for. The allowable number of spore forming cells is <300 spores/$m^2$ and 300,000 spores per spacecraft. Category IVb missions are for lander and probe carrying life detection experiments and require complete system sterilization. A total of 30 organisms including both vegetative and spore forming organisms are allowed per spacecraft. Finally, Category V refers to Earth-returning missions to any solar system body. Depending on the target planet, it can be declared as an "unrestricted Earth return" or strict planetary protection measures may be imposed. More recently, COSPAR defined a new mission category IVc that investigates Martian special regions even if it does not include life detection experiments. In this category, sterilization to Viking post-sterilization biological burden level is required for the entire landed systems.

*Planetary protection state of Practice*

The state of practice for forward protection consists of four major areas: cleaning, sterilization, validation, and recontamination prevention. The current accepted spacecraft cleaning practice used by spacecraft engineers is isopropyl alcohol wiping. The NASA-approved sterilization protocol is dry heat microbial reduction. Although an alternative sterilization method using hydrogen peroxide vapor has been used extensively by the European Space Agency, it is still in the certification phase and has not yet approved by NASA. Validation is conducted according to NASA Procedure and guidelines NPG 8020.12B using NASA standard culture-based assay. Recontamination prevention is achieved by assembling spacecraft in cleanroom environment during assembly, test, and launch operations (ATLO).

*Mars Technology Program Planetary Protection Portfolio*

The Mars Technology Program (MTP) is part of NASA's Mars Exploration Program (MEP). The purpose of MTP is to develop technologies needed for future Mars missions.

The two main program elements of MTP are the Focused Technology and the Base Technology Programs. The Focused Technology Program addresses technologies that are specific and critical to near-term missions, while the Base Technology Program addresses technologies that are applicable to multiple missions and can be characterized as longer-term, higher-risk, and higher-payoff technologies.

Over the years, MTP has funded and managed many planetary protection research tasks in the areas of organic and biological cleaning, enzyme based cleanliness validation methods, biodiversity in spacecraft assembly environments, and sterilization technology development. In this paper, we will introduce seven planetary protection technology development Tasks currently funded and managed by the MTP base technology program. These tasks were selected through NRA 03-OSS-01 process. Most of the tasks are of three years duration starting in August 2004. We will have a brief description of each task and discuss its relevance and status.

## 2. MTP PLANETARY PROTECTION TECHNOLOGY DEVELOPMENT TASKS

*TASK 1. Cleaning to Achieve Sterility*

Current Planetary Protection practice can achieve the Category IVa requirement, which calls for the reduction of viable spore-forming bacteria to 300 spores/$m^2$ or 300,000 spores per spacecraft. But for Category IVb missions, the requirement becomes much more stringent. It allows only 30 viable vegetative and spore-forming bacteria residual per spacecraft. This can be achieved by Viking-type whole system dry heat sterilization, but more sophisticated life detection instruments and electronics may not be able to withstand the high temperature in the dry heat sterilization process. Furthermore, the debris and remnants of the dead cells contain bioorganic matters that if left on the spacecraft after sterilization, may interfere with the life detection science mission. Therefore, an alternative sterilization method is needed. The objective of "Cleaning to Achieve Sterility" is to find an effective cleaning tool to clean spacecraft to <30 viable organisms per spacecraft. This task is lead by PI Roger Kern at Jet Propulsion Laboratory.

In this task, the researchers are evaluating three existing cleaning methods at three facilities:

- Precision Cleaning Operation at JPL
- Ultra Pure Water System, White Sands Testing Facility at JSC
- Liquid Boundary Layer Disruption System (LBLD) at a commercial company

In order to evaluate the cleaning effectiveness of the three systems, 12 spacecraft material coupons are inoculated with *Bacillus Subtilis* bacterial spores. In previous cleaning studies, we deposited spore on the coupons by putting down droplets of spore suspension to the coupons and let them dry for days. The problem with this approach is that the wet deposition and the drying process can alter the adhesion of the spore to the coupons. In this study, the team has developed a spore deposition chamber, which is capable of depositing dried, non-clustered spores to the material coupons. Figure 1 is an illustration of the chamber.

This task has just finished its first year MTP task progress review. The major accomplishments for the first years are:

1. Completed the fabrication and calibration of the aerosol spore deposition chamber.

2. Deposited and calibrated the spore distribution on 12 coupon materials.

3. Evaluated the cleaning effectiveness of the three cleaning systems on removing spores from the coupon surfaces. The results will publish in a separate IEEE paper in this issue.

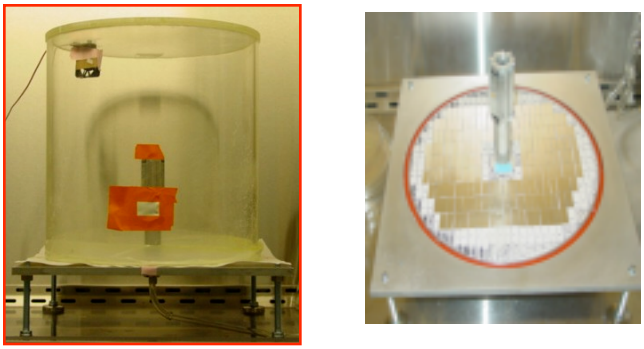


Figure 1. Aerosol spore deposition chamber

At the end of the three-year period this task will deliver a report on the evaluation of cleaning effectiveness of the three cleaning systems. It is expected to recommend a method capable of reducing the current bioburden by four orders of magnitude. If successful, this will advance current cleaning capability and allow us to achieve Category IVb requirement for future missions.

*Task 2. Rapid Single Spore Enumeration Assay*

Currently, bioburden levels on spacecraft are determined using the NASA standard assay, in which bacterial spores are quantified in terms of colony forming units (CFU) that become visible on growth plates after 32$^{o}$C incubation [15]. There are several limitations for the NASA standard assay. First, the process is slow, requiring 3 days to complete. Second, it under-estimates the bioburden by only counting cultivatable spore-forming species which are estimated to constitute less than 1% in soil samples [16-18].

PI Adrian Ponce at JPL is leading a task to develop a rapid single spore enumeration assay (RapidSSEA) that can avoid the limitations of the NASA standard assay. The operational capabilities of RapidSSEA will include (1) rapid enumeration of individual bacterial spores on the timescale of minutes, (2) independence of assay performance from concentration of particulates, and (3) independence of assay performance from cultivability of bacteria. The advantages of RapidSSEA are gained because it is based on a molecular approach. Spore can be considered as a 1-micron diameter bag filled to a very high concentration with a unique chemical marker, dipicolinic acid (DPA). DPA is found only in the core of bacterial spores [18], and can be released from the core into the surrounding volume by inducing germination (e.g., with L-alanine) or physical lysing (e.g., autoclaving, microwaving). When DPA is released into a solution containing terbium ($Tb^{3+}$), highly selective binding of DPA to $Tb^{3+}$ triggers bright green luminescence when viewed under UV light [19, 20]. The unique photophysical properties of $Tb^{3+}$ give rise to an important favorable property. Millisecond $Tb^{3+}$-luminescence lifetimes enable lifetime-gate detection, which effectively removes all background fluorescence (i.e., interferent fluorophores with nanosecond lifetimes), thus rendering the background intensity for imaging silent. Elimination of this background enables a striking increase in image contrast and thus sensitivity for detection. Lifetime gating will enable reproducible single spore enumeration across a wide variety of samples, including those that contain fluorescent interferents frequently found in environmental samples.

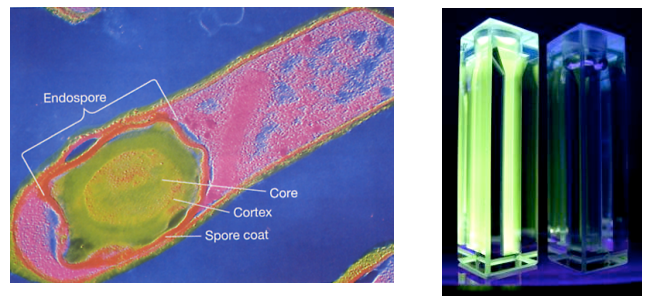



Figure 2. General spore detection strategy via DPA-Tb fluorescence assay.

The accomplishments in the first year are:

1. Built and tested RapidSSEA instrument based on a microscope mounted with a time-gated camera and the method of imaging/counting Tb-DPA luminescence halos surrounding the spore body as DPA is released during spore germination.

2. Demonstrated single spore imaging using RapidSSEA.

3. Correlated RapidSSEA with respect to NASA standard assay for 500,000, 50,000, and 5,000 spores per coupon.

At the end of the third year, a new cleanliness validation method will be reported. The detection sensitivity for viable cultivatable spores is expected to be comparable to the NASA standard assay. The new method will also be able to take into account the viable but non-cultivatable spores on the spacecraft surfaces. The detection time will also be dramatically reduced.

*Task 3. Light Weight Biobarrier Technology*

Planetary protection requirements for a category IVc mission requires the reduction of the bioburden levels for designated portions of the spacecraft beyond the level of the rest of the spacecraft, typically by more than four orders of magnitude. Further, those portions must be protected from recontamination until the spacecraft reaches its target

location, and possibly during surface operations. Such biobarriers must be compatible with selected microbial reduction methods and must also allow the enclosed hardware to deploy without interference.

PI Yuki Salinas and her team at JPL are developing a lightweight biobarrier that will maintain the sterility of an IVc subsystem. There are two objectives of this task: 1) to provide a biobarrier material and structure which enables a subsystem to meet the planetary protection bioburden requirements of Category IV-b equivalent: 2) to provide a fail-safe deployment mechanism such that the sample acquisition device and/or the sample handling/transfer device (i.e., part of the spacecraft which is designated Category IV-b equivalent) functions without interference of the bio-barrier structure.

During the first year, Yuki's team has completed manufacturing and extensive material testing of a proto-type biobarrier compatible with dry heat microbial reduction, using DuPont Tedlar as the biobarrier material. It is a clam-shell design with spring-loaded ribs that is deployed by a single pinpuller. Integrated HEPA filters allow for launch and EDL pressurization. The design was successfully adopted for use in the Phoenix Robotic Arm assembly to meet the Phoenix planetary protection requirement.

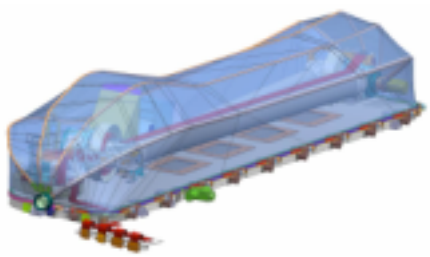


Figure 3. Biobarrier on a robotic arm

The following is the summary of the first year accomplishments of the task:

1. Completed the biobarrier material selection and testing.

2. Completed the prototype biobarrier fabrication and deployment testing.

3. Conducted seal effective test and temperature and hydrogen peroxide test on the materials.

At the end of the task period, the project will deliver the biobarrier technology and systems to protect portions (subsystems) of a spacecraft from recontamination until the subsystem is ready to deploy on Martian surface. It will also establish microbial reduction methods that are compatible with both dry heat microbial reduction and $H_2O_2$ sterilization protocols.

*Task 4. Spore Adhesion for Contamination Transport Models*

In the field of contamination control (rather than planetary protection), most well-developed transport models available in the literature pertain to particulate contamination, and virtually none to biological contamination. For planetary protection needs, particulate transport models offer the most reasonable approach to the development of biological contamination transport models. Spores are particles, and most spores are believed to be associated with particles. In order to apply particle transport models to planetary protection, one must know the average number of spores per particle in a range of sizes of interest, including fractions of small particles that are spores. Also, the knowledge of the physical relationship between biological contamination (spores) and airborne and spacecraft surface particulates is needed for realistic biological contamination transport models. Specifically, the adhesion forces among these contaminants are critical in understanding and describing these relationships. Removal of a contaminant particle by environmental conditions that overcome the adhesion forces is a critical step in the particle's transport. This investigation will address both aforementioned issues: the number of spores associated with particles as a function of particle size and the adhesion forces. The transport models in turn provide validations of terrestrial contamination transport simulation and independent predictions of biological contaminant transport before, during, and after launch.

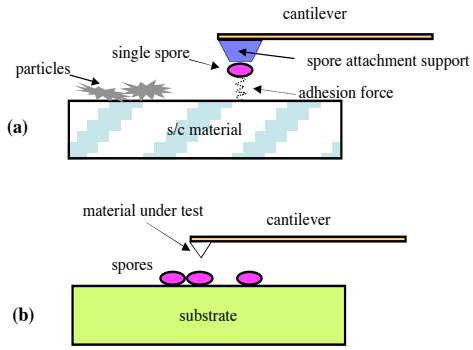
PI Ying Lin at JPL is leading the task to develop a method to understand spore association with dust particulates in a clean room environment by measuring the number of spores on particles of various sizes and to investigate spore adhesion to its carriers by AFM measurements of adhesion forces among spores, particles, and spacecraft material surfaces. The results of this investigation will improve our understanding of spacecraft material cleanability at a fundamental level. The direct measurement of adhesion forces between biological particulates and variety of spacecraft materials will provide valuable information on the forces required to remove particles from various spacecraft materials [21]. This data will help in PP cleaning, material selection, contamination risk assessment, and developing optimal spacecraft cleaning strategies.

The following is the first year accomplishment of the task:

1. Completed indoor and outdoor air sampling study and established statistic quantification on how many spore-forming bacteria associating with what size of particles in air.

2. Developed a methodology to attach single spore to a cantilever of atomic force microscope.

3. Characterized the spacecraft material coupon surface




morphology, roughness and chemical variation.

Figure 4. Spore adhesion force measurements

At the end of this work, the task will deliver a final report on bacterial spore association with dust particle on spacecraft surfaces in the clean room environment. It will also make a quantitative assessment of bacterial spore attachment/detachment efficiency to and from spacecraft surfaces. This knowledge is essential for the development of bio-contamination transport models, which can be used to determine the forward cross-contamination risks for MEP missions.

*Task 5. Near Field and Integrated Particle Transport Model*

This task aims to develop a computer simulation model to describe the near field particle transport process under Martian conditions. It will provide analysis of the probability of particle entrainment from spacecraft surfaces to the landing site on Mars. Contamination transport model from point and line sources in a uniform wind is currently available. This model can be used to estimate concentrations of contamination at large distances (100 m to 1 km) from a lander. For applications involving sampling near a lander, point and line sources are very crude. A near field particle transport model must be developed to estimate the cross contamination risk at the landing and sampling sites. PI Partha Shakkottai at JPL is leading an effort to develop a computer simulation program that shows how particles from spacecraft are transported by Martian winds. This model will take into account of the influences on particle sizes, wind variability, particle adhesion, and geometry of the spacecraft. The experimental validation of the model is conducted at the Arizona State University and at NASA Ames using wind tunnels.

The following are the first-year accomplishments of the task:

1. Selected GEANT4 as the base particle transport model.

2. Particle motion in a uniform wind was demonstrated on an elementary model.

3. Conducted preliminary wind tunnel tests (ASU and NASA Ames).

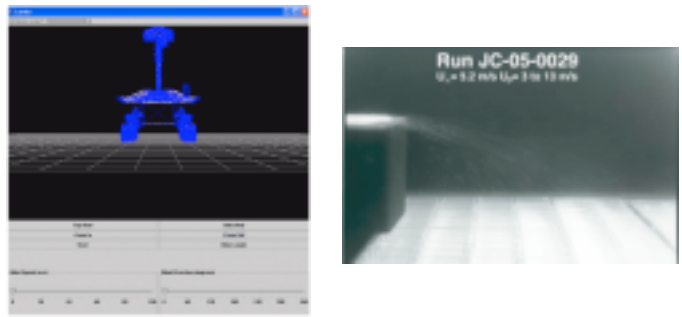



Figure 5. Particles on a sample spacecraft and experimental testing

At the end of the third year, this task promises to deliver a computer simulation model. Planetary protection engineers can use this model to determine the probability of particle contamination of the landing site when the input conditions such as particle size and wind speed and direction are given.

*Task 6. Mars Orbital Debris Analysis Tool*

PI Walt Bruce at NASA Langley Research Center is leading an effort to develop a thermal analysis tool that can be used to quickly determine if small debris will meet planetary protection sterilization requirements at any point along a breakup trajectory.

The planetary material entering an extraterrestrial solar system body must reach a specified temperature for a specific period of time to be considered sterile. Presently, this requirement is 500°C for 0.5 seconds. Even missions planned as orbital or fly-by missions must address planetary protection requirements in the event the spacecraft inadvertently enters the Martian atmosphere and breaks up during entry resulting in the debris either impacting the surface, burning-up in the atmosphere or a combination of the two. If a mission can show through analysis that debris from a Martian entry will meet or exceed the thermal planetary protection requirements, then the cleaning and sterilization efforts prior to launch may be substantially reduced or eliminated. This can result in a substantial cost and schedule savings prior to launch.

The increasing number of future Martian missions and the requirement to assess the bioburden of each mission necessitates the development of an analysis tool that can be used to quickly and easily evaluate the Martian planetary protection requirement for small particle debris. As a satellite enters the Martian atmosphere and starts to break-up, small particles can be released into the atmosphere at various and numerous points along the break-up trajectory. Large portions of the satellite may break apart and assume separate trajectories with different velocities and entry angles. At each of these major breakup points many smaller

pieces of debris, which were previously shielded from the aerothermal heat loads, can be introduced into the flow stream. An analysis tool is needed to quickly assess which size particles and materials will meet the planetary protection requirements based on the new trajectory variables. Typically, a rather exhaustive analysis is performed to evaluate the breakup and burn-up of the larger items and components on the satellite; however, smaller components are often assumed to burn-up in the atmosphere. This is potentially a reasonable assumption for Earth entry; however, a study of small meteorites entering the Martian atmosphere suggests that small particles may not burn-up and demise prior to impacting the surface [22]. Because of this data it is imperative that small particles are evaluated during a burnup/breakup analysis to ensure they meet the planetary protection requirements and to add robustness to the final planetary protection analysis. Even if the small debris is considered, it is typically analyzed using a process that involves several codes. Currently, there is no one code available that specifically addresses planetary protection issues for Mars. Typically, these codes are rather complicated and are very comprehensive to cover many situations and types of analysis. These codes have many inputs and requirements that make it difficult for an occasional user to quickly pickup and use the code with confidence.

The LRC team has developed an analysis tool for small debris to support the breakup/burn-up analysis for the Mars Reconnaissance Orbiter at the request of JPL. This tool has proven to be very useful for providing results for planetary protection issues for small debris. However, this tool has many assumptions that need improvement to enhance the quality of the code. Also, this code is presently in an Excel Spreadsheet format and we would like to develop it into a FORTRAN executable code. This task is to continue development of this tool to eliminate some of the less accurate engineering assumptions that are currently in the model replacing them with higher-level models that can be found in the literature. Another improvement is to include options for various materials and Martian atmosphere models. Another goal of the task is to put the code in a user-friendly executable format so that it is easy and quick to run. We will also validate or verify the code data against other analyses processes and data available in the literature.

This is a two-year task. This task is currently behind schedule due to the activity PI had in supporting the shuttle program. Several subtasks have just started and have not yet achieved significant accomplishment.

At the end of the program, the task promises a thermal analysis tool that can be used to quickly determine if small spacecraft debris will meet the planetary protection sterilization requirements at any point along a breakup trajectory. Several inputs will be required such as particle size, velocity, initial altitude, and flight angle relative to the Martian surface. A menu selection will be available to pick the material and atmosphere desired or a custom material can be input. The code will be very user friendly with minimal input requirements with a specific purpose of addressing planetary protection requirements entering the Martian atmosphere.

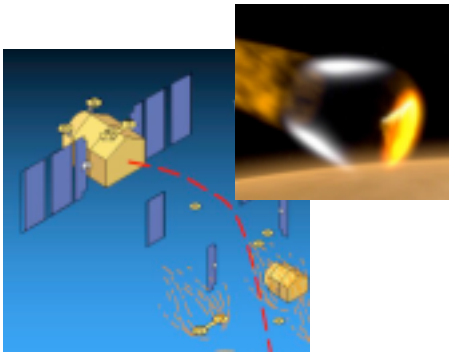


Figure 6. Mars Orbital debris

*Task 7. Contained Sample Handling and Analysis*

One of the cornerstones of NASA's long-range program of planetary exploration is to return a scientifically rich sample of Mars rock, regolith, and atmosphere to Earth. Once the sample returns to Earth, it must be treated as biologically active and hazardous until proven to be safe. Although still in the earliest stages of planning, it has been recognized that some facility must be provided to both protect the Earth from the sample (biohazard containment) and also to protect the sample from Earth (contamination prevention). Because of the scientific significance of the sample and the aforementioned public safety implications, efforts are already underway to develop a protocol for the handling and analysis of the returned sample.

A NASA-developed protocol for Mars returned sample handling exists today in a draft form. It has been a consistent theme during the development of the protocol that some effort must be made to analyze the physical and chemical characteristics of the sample as a prelude to life detection and biohazard testing and subsequent release to the broader community for scientific analysis. The specific methodology for this analysis has not yet been defined, and no existing apparatus or protocol provides for both sample-to-environment and environment-to-sample isolation. When providing for isolation and containment of a potential biohazard, it is important to minimize the interactions between humans and the sample — not just for the protection of those interacting with the sample, but also because the most common causes of containment breaches tend to be associated with human handling. The ideal system would provide both sample-to-environment and environment-to-sample protection, largely or totally autonomous sample handling and analysis, and extensive analytical functions. This task is intended to deliver a system exhibiting exactly these needed capabilities, dramatically reducing the parallel risks of inadvertent loss of Mars sample containment and sample contamination.

PI Joe Parrish at Payload Systems Inc. is developing a new returned-sample handling instrument for handling and

analysis of a subset (e.g., fine particles) of the returned sample. This instrument will be based on the existing Cell Culture Unit (CCU) which is an autonomous bioreactor habitat being developed by Payload Systems Inc. for NASA.

The CCU has several core features — including multiple levels of sample segregation and containment, reagent addition/extraction, automated sub-sampling, video microscopy, thermal conditioning, precise environmental sensing and control, etc. that are applicable for use in returned Mars sample handling and analysis. It is well-suited for handling and analysis of solid "fines" (unconsolidated regolith, atmospheric dust, and dust generated from coring operations). The approach is to adapt the CCU flight system technologies into a Contained Sample Handling and Analysis System (CSHAS) for use on Earth to perform analysis, testing, preparation and other functions in support of defined Mars returned sample handling and analysis protocols

The following are the first year accomplishments of the task:

1. Defined the CSHAS role in SRF: Resulted in the CSHAS Functional Requirements Document.

2. Defined a sample handling architecture.

3. Assessed the feasibility of CCU to contribute to the CSHAS design: Resulted in the CCU Feasibility Assessment Document.

At the end of three years, the team will have developed a Contained Sample Handling and Analysis System (CSHAS) for use on earth to perform analysis, testing, preparation and other functions in support of the defined Mars returned sample handling and analysis protocol.

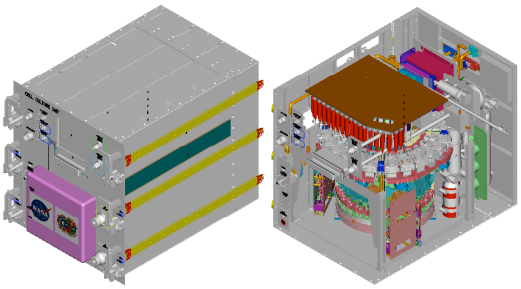


Figure 7. Existing Cell Culture Unit (CCU)

## 3. CONCLUSION

The goal of planetary protection is to preserve the biological and organic conditions of solar system bodies for future exploration while at the same time, protecting the Earth from potential extraterrestrial contamination. Planetary protection requirements for each mission will be based on the type of encounter it will have (flyby, orbiter, or lander) and the potential of the mission's destination to provide insight into the origin of life. To implement planetary protection requirements, we need to advance current technologies to satisfy planetary protection requirements for surface, subsurface and atmospheric missions as well as those technologies that allow sample acquisition for in-situ life detection or sample return. These technologies include cross-contamination risk assessment and prevention; pre-launch bioburden reduction and validation; heating of orbital debris during atmospheric entry at Mars; and sample return issues including containment, handling and sample analysis.

We have discussed the seven Planetary Protection technology tasks managed by Mars Technology Program. These tasks cover a wide range of planetary protection technology development strategies envisioned by NASA. The successful completion of these tasks will provide new or improved capabilities to meet planetary protection requirements for future missions.

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## BIOGRAPHY

**Dr. Ying Lin** is a senior member technical staff at JPL's biotechnology and planetary protection group. She has a BS in chemical engineering from Tsinghua University and a Ph.D. in Chemistry from University of Arizona. She has extensive research experience in chemistry, biochemistry and chemical engineering. She is the PI and Co-I of several proposals funded by JPL and NASA on planetary protection. She is currently a technical lead for the planetary protection tasks for Mars Technology Program.

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