Particulate Removal Using a CO_2 Composite Spray Cleaning System

Nicole Chen
Jet Propulsion Laboratory, California Institute of Technology
4800 Oak Grove Dr.
Pasadena, CA 91109
818-354-4542
Nicole.C.Chen@jpl.nasa.gov

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

David Jackson
Clean Imagineering LLC
26074 Ave Hall Unit 6
Santa Clarita, Ca 91355
661-430-6768
David.jackson@cleanlogix.com

Shirley Chung
Jet Propulsion Laboratory, California Institute of Technology
4800 Oak Grove Dr.
Pasadena, CA 91109
818-354-4005
Shirley.Y.Chung@jpl.nasa.gov

Abstract— **Abstract -The Planetary Protection surface cleanliness requirements for potential Mars Sample Return hardware that would come in contact with Martian samples may be stricter than previous missions. The Jet Propulsion Laboratory has developed a new technology that will enable us to remove sub-micron size particles from critical hardware surfaces. A hand-held CO_2 composite cleaning system was tested to verify its cleaning capabilities. This convenient, portable device can be used in cleanrooms for cleaning after rework or during spacecraft integration and assembly. It is environmentally safe and easy to use. This cleaning concept has the potential to be further developed into a robotic cleaning device on a Mars Lander to be used to clean sample acquisition or sample handling devices *in situ*. Contaminants of known sizes and concentrations, such as fluorescent microspheres and spores were deposited on common spacecraft material surfaces. The cleaning efficiency results will be presented and discussed.**

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I. INTRODUCTION

Planetary protection and contamination control are integral parts of all NASA space exploration missions. Their primary objectives are to reduce the risk of Earth-based contaminants cross-contaminating extraterrestrial samples and to ensure mission success. Component and system level sterilization methods such as dry heat microbial reduction and hydrogen peroxide sterilizations require enclosed chambers with stringent environmental parameter control and spatial limitations. These methods are effective in killing live microorganisms but remnants remain on the surfaces. The remnants contain thousands of molecules that could contaminate extraterrestrial samples.

Current accepted spacecraft-compatible cleaning protocols involve vapor degreasing, liquid sonication, and alcohol wiping. However, a recent study (Chen, 2007) shows that these methods are not fully effective in removing live and dead microbes from spacecraft piece parts of slightly complicated geometries such as tubing and nuts and bolts. None of the current accepted methods addresses particulate contamination. As such, a new cleaning method must be developed to address these issues.

A reliable and efficient pre-flight cleaning protocol is a critically important step in achieving the primary planetary protection and contamination control objectives. CO_2 composite spray technology is a safe and convenient method of cleaning that addresses the shortcomings of current cleaning methods. We have collaborated with Cleanlogix LLC to develop a specialized CO_2 composite spray device for microbial and submicron particle removal. The objective of this study is to evaluate the cleaning effectiveness of the CO_2 composite spray system and to develop a standardized procedure that optimizes its cleaning capabilities. If successful, this CO_2 cleaning method could potentially be developed and adapted to clean sensitive spacecraft hardware such as exposed optics.

II. BACKGROUND

The CO_2 composite spray cleaning process is analogous to both conventional solvent spray cleaning and ultrasonic immersion cleaning. CO_2 exhibits halogenated solvent-like chemistry and the CO_2 composite spray process delivers powerful pulsating shear stress to surfaces being cleaned. The application of dilute (lean) CO_2 particles-gas spray compositions having variable velocity produce unique phase-change (solid→liquid phase) cleaning action at the substrate surface. Solid-to-liquid phase flushing of

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the substrate surface washes contaminants from the most complex topography, while unique chemistry and precise process control protect delicate surface features from damage.

Based on both chemical and physical considerations, the CO_2 composite spray cleaning process is analogous to the high-pressure Freon 113 spray systems employed prior to the CFC phase-out in the early 1990's. In addition to this, CO_2 composite spray technology provides significant environmental, health, and safety advantages.

Conventional cryogenic spray cleaning processes have traditionally employed supersonic DeLaval-type (convergent-divergent) spray nozzles. The main disadvantage of DeLaval cryogenic spray nozzles is that there is an unbalancing effect at the nozzle exit of the fluid stream. As shown in Figure 1, the surrounding fluid (ambient atmosphere) tends to drag the nozzle fluid stream, causing the flow stream to diverge rapidly upon discharge from the nozzle exit. This causes the liquid droplets or sublimable solid particles to expand quickly, resulting in a significant loss of cleaning agent (solid particles) through plume expansion or the production of numerous and small solid particles, which generally requires the spray nozzle to be placed in close proximity to a substrate surface to be effective.

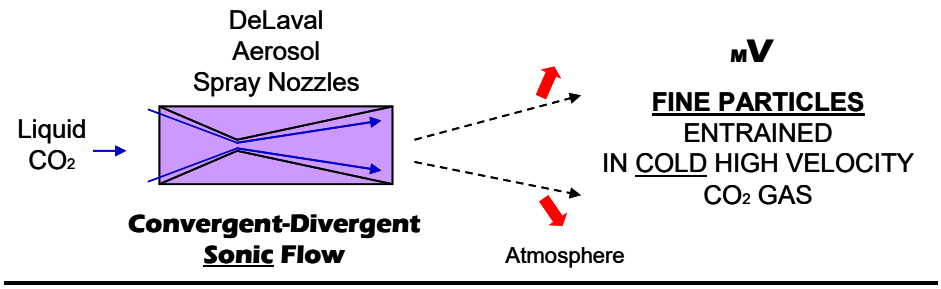


Figure 1: Conventional CO_2 "snow" spray

Shown in Figure 2, a more recent dry spray cleaning improvement which addresses many of the shortcomings of conventional "snow" spray systems in general, and the DeLaval CO_2 spray nozzle design in particular, is a CO_2 composite spray. A CO_2 composite spray is generated using Coaxial or Coaxial-Coanda two-phase composite spray nozzle designs with integrated capillary condensers (CleanLogix 2011). Coaxial composite sprays efficiently produce cleaning sprays containing solid CO_2 particles (cleaning agent) of controllable size, density, concentration, heat capacity, and kinetic energy. To make solid carbon dioxide particles, a capillary condenser assembly comprising an elongated segment of thermally-insulated polyetheretherketone (PEEK) capillary tubing is used. A capillary condenser assembly provides an efficient means for subcooling (boiling) and condensing small amounts of liquid carbon dioxide into a low velocity but dense mass of solid-phase particles. Varying the length and internal diameter(s) of the capillary condenser, including stepping, produces particles having different particle size distribution ranges and density. Once formed, CO_2 particles are injected and vortically mixed into a heated propellant gas such as nitrogen or clean-dry air (CDA), which flows coaxially with the capillary condenser assembly.

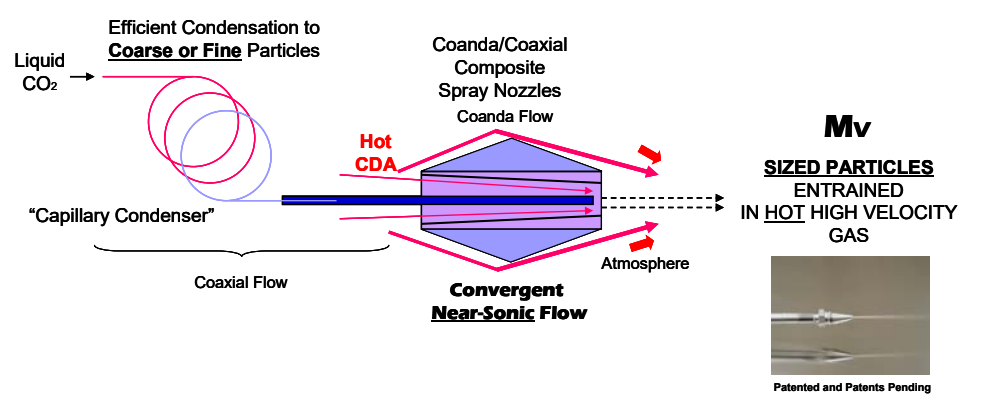


Figure 2: CO_2 composite spray

III. METHOD

Materials

In order to test the cleaning efficiency of the CO_2 composite spray, various substrates and particle contaminants were used. The coupon substrates tested include nitinol, aluminum Al 6061 (an aluminum alloy commonly used for spacecraft hardware), titanium, and stainless steel. The aluminum coupons had dimensions 2.22cm by 0.95cm by 0.079cm while the other materials had dimensions 2cm by 1cm by 0.2cm. For spore testing, Al6061 substrates had surface roughness of 5 and 2 rms (root mean square) microinches. Similarly, for spore testing, nitinol substrates with rms 14 and rms 3 values were tested. Some 3-dimensional substrates are tested as well. These include stainless steel bolts, stainless steel nuts, and spacecraft hardware nitinol plugs of varying designs.

The contaminants used include Bacillus Atrophaeus bacterial spores, 1.0µm diameter FluoSpheres® Aldehyde-Sulfate Microspheres, 0.5µm diameter FluoSpheres® Carboxylate Modified Microspheres, and 0.2µm FluoSpheres® Sulfate Microspheres.

Sample Preparation

Sample substrates were cleaned prior to particle deposition. Coupons would be soaked in acetone for a minimum of 5 minutes in a fume hood. Following this, sample substrates would then be manually cleaned by acetone and isopropyl alcohol wiping. Then the substrates would be rinsed with acetone. Substrates that would undergo spore deposition were also UV sterilized on both sides for 15 minutes each. Once cleaned, the substrates were ready for particle deposition and were placed in sterile petri dishes and sealed with parafilm. Particles were deposited onto the cleaned substrates either through aerosol deposition (dry deposition) or droplet deposition (wet deposition).

Three-dimensional parts were pre-cleaned by 5 minute sonication in acetone, followed by 5 minute sonication in isopropyl alcohol. After sonication, the parts were then placed into pyrex petri dishes and sterilized in an oven by heating to 175°C for 3 hours. The 3-dimensional parts were only used in spore aerosol deposition testing.

With aerosol deposition, the contaminant particles were gently deposited onto the substrate surface. This was accomplished by using an aerosolizer shown in Figure 3. The coupon and 3-dimensional parts placement within the aerosolizer unit is shown in Figure 4. The goal was to deposit 10^5 particles onto the coupon surface. This deposition method simulates natural fallout onto the substrate surface.

Figure 3: Aerosolizer set up used for aerosol deposition

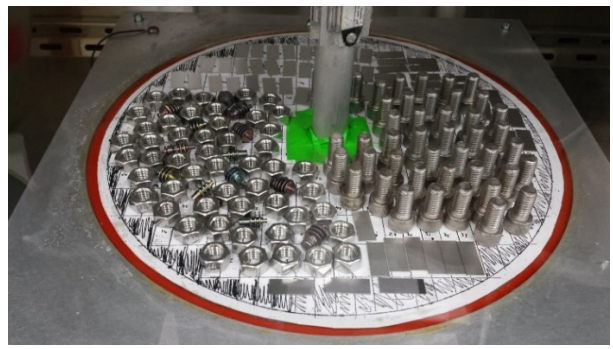


Figure 4: Close up of samples within aerosolizer deposition area

With droplet deposition, the contaminant particles were deposited directly onto the substrate surface as show in Figure 5. A concentration of contaminant particles is created by dilution. Spores were diluted with water. Concentrations used ranged from 10^3 to 10^6 particles per coupon. Fluorescent particles were diluted with water and ethanol in order to ensure a uniform distribution of particles. Dilutions were vortex mixed and sonicated prior to deposition. A 10µL volume was then deposited directly onto the substrate using a pipette. The samples were then left to dry overnight in a Class 100 laminar flow hood.

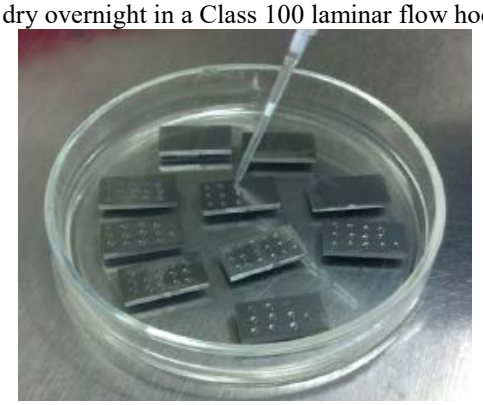


Figure 5: Droplet deposition onto nitinol coupon

CO_2 Cleaning

The CO_2 composite spray device is set up in a cleanroom lab as shown in Figure 6. The device is attached to a N_2 gas cylinder as well as a CO_2 gas cylinder that is connected via a purifier unit. The handheld unit fits within a HEPA-filtered enclosure. The unit is controlled using a foot pedal.

Figure 6: CO_2 composite spray system set up

Although the CO_2 composite spray technology has already been developed at Cleanlogix LLC, it needs to be modified and optimized in order to be more effective for removing our target contaminant. To determine the optimal operating conditions the following key test parameters of the CO_2 device, shown in Figure 7, were varied: angle of incidence, propellant (nitrogen) temperature and pressure, number of passes, spray pattern and the CO_2 spray speed. The ideal parameters we found are propellant (N_2) temperature 130°C, 40 psi propellant (N_2), CO_2 speed ~ 1.3 – 1.5 lbs. of CO_2 per hour, 45° incident to the sample surface, 0.5 inch distance from the sample surface, and 3 passes unidirectional passes across the length and width of the sample surface. All testing and cleaning were done in triplicate.

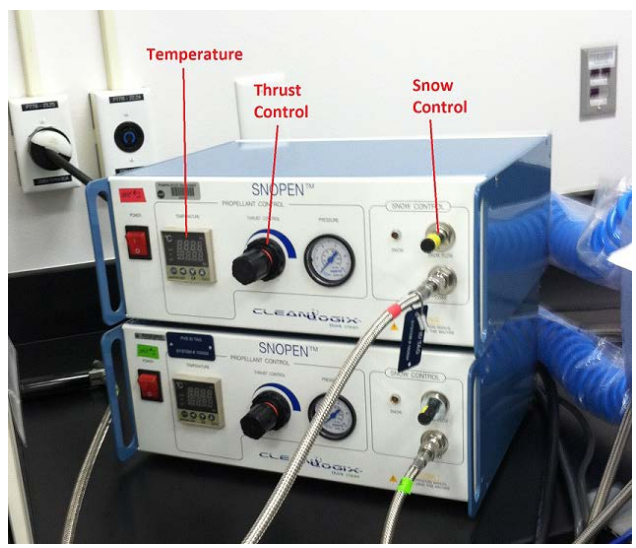


Figure 7: Close up of CO_2 composite spray unit showing adjustable parameters

Spore Analysis

Spore samples were analyzed using the NASA standard assay procedures. After cleaning, the sample coupons were placed in individual sterile glass test tubes with 10mL of sterile water. The samples were then vortex mixed and sonicated to release remaining spores. Then 2mL of each

sample tube was plated in tryptic soy agar (TSA), four plates per sample. The plated samples were then incubated at 32°C. Spore colonies, if any, were counted 24 and 48 hours after incubation.

If there are no spore colonies after 48 hours of incubation, then 10mL of sterile tryptic soy broth (TSB) is added to the test tube containing the cleaned sample. This is done to test for sterility. After 7 days of incubation at 32°C, if the coupon is sterile, then the broth will remain clear. However, if a single microbe is in the tube, regardless of origin, the sterility test will fail.

Fluorescent Particle Analysis

After cleaning, fluorescent particle samples were analyzed using a Zeiss fluorescent microscope. The microscope set up is shown in Figure 8.

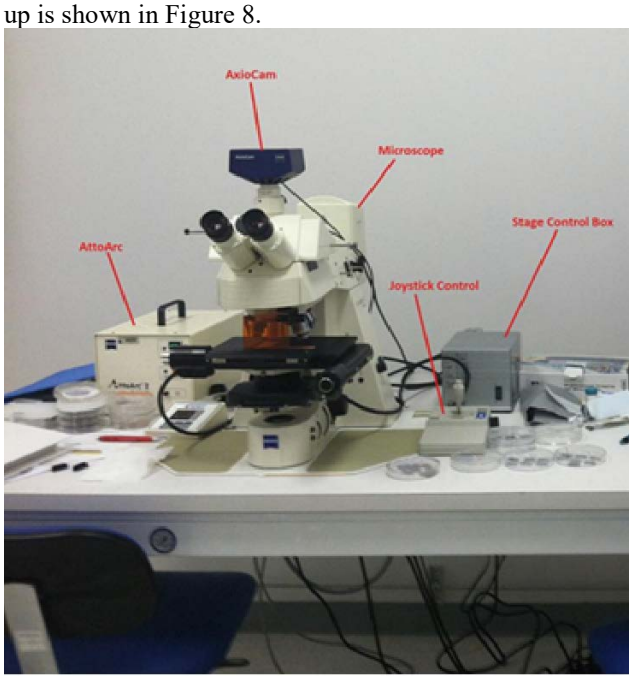


Figure 8: Fluorescent microscope set up

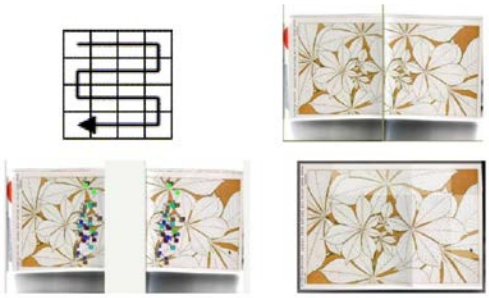


Figure 9: Example of scanning raster pattern and stitching methodology

Sample substrates were placed onto the moving stage and a raster scan was performed across the sample surface. Images are taken of sections of the sample surface and stitched together to form a complete image of the entire surface. This image was acquired through the Axiovision program. An example of the raster pattern and stitching method are shown in Figure 9. The scanned image was then processed using ImageJ to count the particles. Figure 10 shows an image of a sample that was scanned by the Zeiss fluorescent microscope and analyzed in ImageJ before CO_2 cleaning. Similarly, Figure 11 shows an image of a sample that was scanned by the Zeiss fluorescent microscope and analyzed in ImageJ after CO_2 cleaning

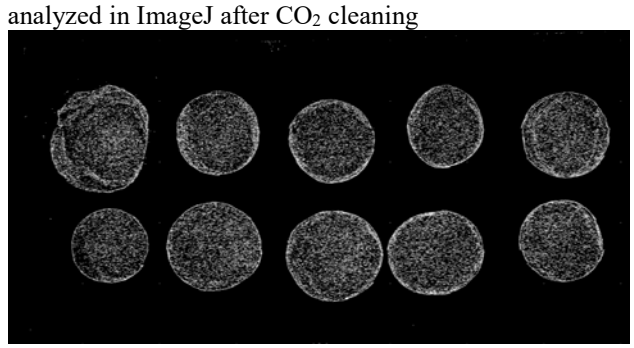


Figure 10: Image of sample before cleaning 0.5µm droplet deposition on a nitinol coupon analyzed in ImageJ

Figure 11: Image of sample after cleaning 0.5µm droplet deposition on a nitinol coupon analyzed in ImageJ

IV. RESULTS

The cleaning results are listed below in Tables 1 – 7. These tables show the cleaning results after using the optimal process parameters. The optimal process parameters are propellant (N_2) temperature 130°C, 40 psi propellant (N_2), CO_2 speed ~ 1.3 – 1.5 lbs of CO_2 per hour, 45° incident to the sample surface, 0.5 inch distance from the sample surface, and 3 passes unidirectional passes across the length and width of the sample surface. All testing and cleaning were done in triplicate. Spore data is calculated by counting the number of colony forming units (cfu) before and after CO_2 cleaning. Similarly, fluorescent particle data is calculated by counting the number of particles before and after CO_2 cleaning.

As seen in Tables 1 – 3, the CO_2 device is capable of cleaning to sterility for aerosol deposited spores and is capable of cleaning a minimum of a 4-log reduction for droplet deposited spores. This minimum 4-log reduction matches current dry heat microbial reduction procedures. These results are shown in Figure 12.

Table 1: Spore cleaning data on Al6061

Spore cleaning data on Al6061				
Substrate	**Deposition Method**	**Before (cfu)**	**After (cfu)**	**Log Reduction**
Al6061 rms 5.5	Aerosol	1.36E+05	6	4.34
		1.36E+05	4	4.56
		1.36E+05	0	sterile
Al6061 rms 5.5	Droplet	1.58E+06	90	4.24
		1.58E+06	3	5.80
		1.58E+06	20	4.90
Al6061 rms 2	Aerosol	2.91E+06	3	6.07
		2.91E+06	3	6.07
		2.91E+06	8	5.59
Al6061 rms 2	Droplet	7.13E+05	44	4.21
		7.13E+05	20	4.55
		7.13E+05	33	4.34
Anodized clear	Aerosol	3.21E+06	43	4.88
		3.21E+06	55	4.77
		3.21E+06	26	5.09
Anodized clear	Droplet	7.71E+05	53	4.17
		7.71E+05	23	4.53
		7.71E+05	28	4.45

Table 2: Spore cleaning data on nitinol

Spore cleaning data on nitinol				
Substrate	**Deposition Method**	**Before (cfu)**	**After (cfu)**	**Log Reduction**
Nitinol rms 14	Aerosol	8.93E+05	0	Sterile
		8.93E+05	0	Sterile
		8.93E+05	0	Sterile
Nitinol rms 14	Droplet	2.32E+06	0	Not sterile
		2.32E+06	0	Not sterile
		2.32E+06	1	6.27
Nitinol rms 3	Droplet	1.04E+06	1	5.92
		1.04E+06	0	Sterile
		1.04E+06	0	Sterile

Table 3: Spore cleaning data on titanium

Spore cleaning data on titanium				
Substrate	**Deposition Method**	**Before (cfu)**	**After (cfu)**	**Log Reduction**
Titanium rms 16	Aerosol	2.58E+05	0	Not sterile
		2.58E+05	0	Not sterile
		2.58E+05	0	Not sterile
Titanium rms 16	Droplet	2.56E+06	3	6.01
		2.56E+06	18	5.16
		2.56E+06	36	4.85
Titanium rms 32	Aerosol	3.19E+05	1	5.41
		3.19E+05	0	Not sterile
		3.19E+05	0	Not sterile
Titanium rms 32	Droplet	2.24E+06	33	4.84
		2.24E+06	58	4.59
		2.24E+06	0	Not sterile
Titanium rms 64	Aerosol	3.13E+05	0	Not sterile
		3.13E+05	0	Not sterile
		3.13E+05	1	5.40
Titanium rms 64	Droplet	2.70E+06	94	4.46
		2.70E+06	11	5.38
		2.70E+06	201	4.13

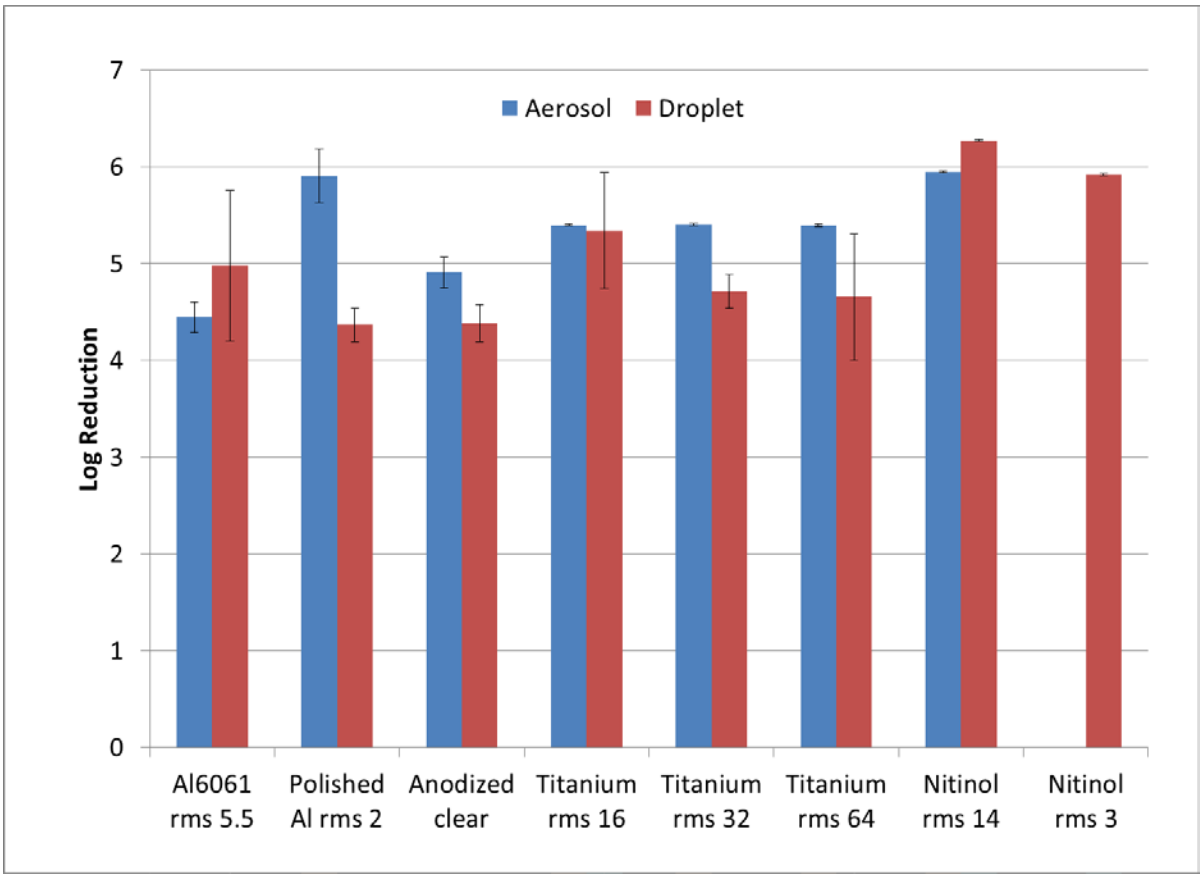


Figure 12: Spore analysis, log reduction results summary after cleaning with optimal process parameters

As can be seen in Table 4, our device is at best, capable of cleaning 3-dimensional substrates with complex geometries. The CO_2 composite spray unit is capable of a minimum of a 4-log reduction in aerosol deposited spores and a maximum of sterility for such substrates. These results are illustrated in Figure 13.

Table 4: Spore cleaning data on 3-dimensional parts

Spore cleaning data on 3-dimensional parts				
Substrate	**Deposition Method**	**Before (cfu)**	**After (cfu)**	**Log Reduction**
Stainless steel bolts	Aerosol	3.23E+05	5	4.81
		3.23E+05	5	4.81
		3.23E+05	4	4.94
		3.23E+05	11	4.46
		3.23E+05	4	4.94
		3.23E+05	14	4.37
Stainless steel nuts	Aerosol	1.33E+05	6	4.35
		1.33E+05	0	Not sterile
		1.33E+05	1	5.03
		1.33E+05	0	Not sterile
		1.33E+05	0	Not sterile
		1.33E+05	4	4.52
Nitinol plug – old design	Aerosol	1.29E+05	0	Sterile
		1.29E+05	0	Not sterile
		1.29E+05	5	4.41
Nitinol plug – new design	Aerosol	1.17E+05	0	Sterile
		1.17E+05	0	Sterile
		1.17E+05	1	5.07

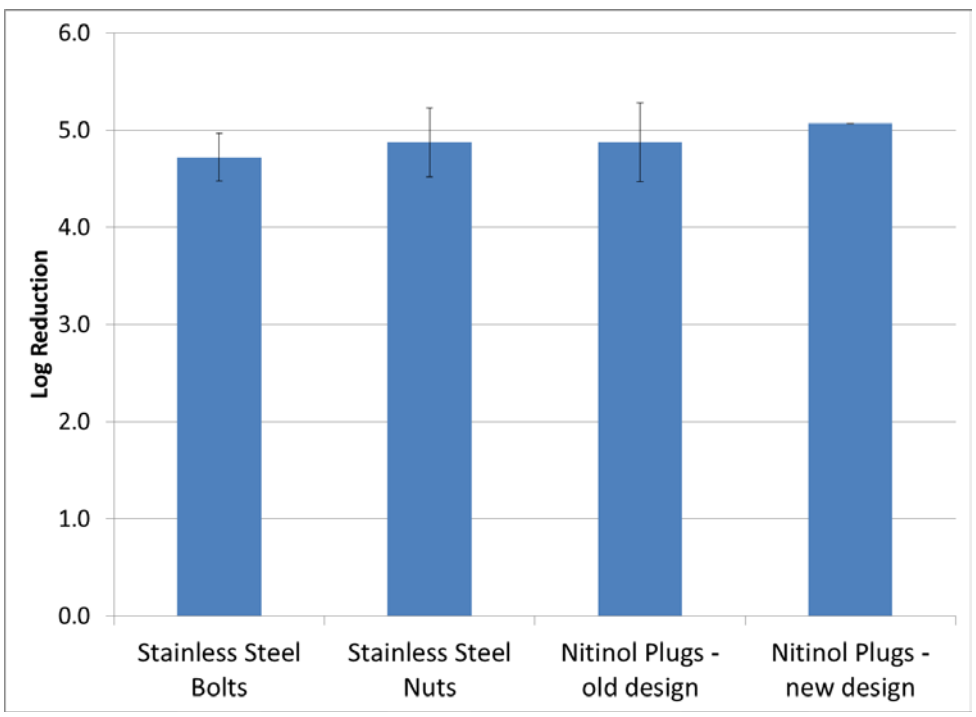


Figure 13: Spore analysis, log reduction results summary after cleaning with optimal process parameters on 3-dimensional parts

As can be seen in Tables 5 – 7, our device is able to remove most aerosol deposited submicron particles for a minimum of a 4-log reduction. This minimum 4-log reduction matches current dry heat microbial reduction process. We were able to remove up to a 2-log reduction for droplet deposited submicron particles. These results are summarized in Figure 14 above.

Table 5: 1µm fluorescent particle cleaning data

1.0µm fluorescent particle cleaning data				
Substrate	**Deposition Method**	**Before (# of particles)**	**After (# of particles)**	**Log Reduction**
Titanium rms 3	Aerosol	7.64E+04	8	4.28
		9.93E+04	5	5.00
		7.31E+04	1	3.96
Stainless steel rms 3	Aerosol	9.55E+04	4	3.57
		9.25E+04	0	4.19
		9.46E+04	8	4.07
Nitinol rms 3	Aerosol	9.54E+04	26	4.08
		6.90E+04	6	4.14
		7.79E+04	8	4.89
Nitinol rms 3	Droplet	1.39E+03	196	0.85
		2.43E+03	261	0.97
		1.42E+03	150	0.98
		7.66E+03	32	2.38
		8.17E+03	4	3.31
		7.59E+03	50	2.18
		4.47E+04	132	2.53
		5.10E+04	691	1.87
		4.94E+04	193	2.41

Table 6: 0.5µm fluorescent particle cleaning data

0.5µm fluorescent particle cleaning data				
Substrate	**Deposition Method**	**Before (# of particles)**	**After (# of particles)**	**Log Reduction**
Nitinol rms 3	Aerosol	1.33E+05	0	5.12
		1.30E+05	2	4.81
		1.21E+05	0	5.08
Nitinol rms 3	Droplet	1.03E+03	83	1.10
		1.30E+03	116	0.95
		9.30+02	105	0.95
		7.68E+03	185	1.62
		7.56E+03	185	1.61
		7.22E+03	307	1.37
		3.09E+04	333	1.97
		4.48E+04	1411	1.50
		4.56E+04	1045	1.64

Table 7: 0.2µm fluorescent particle cleaning data

0.2µm fluorescent particle cleaning data				
Substrate	**Deposition Method**	**Before (# of particles)**	**After (# of particles)**	**Log Reduction**
Nitinol rms 3	Aerosol	3.43E+05	20	4.23
		1.82E+05	11	4.22
		1.39+05	5	4.44

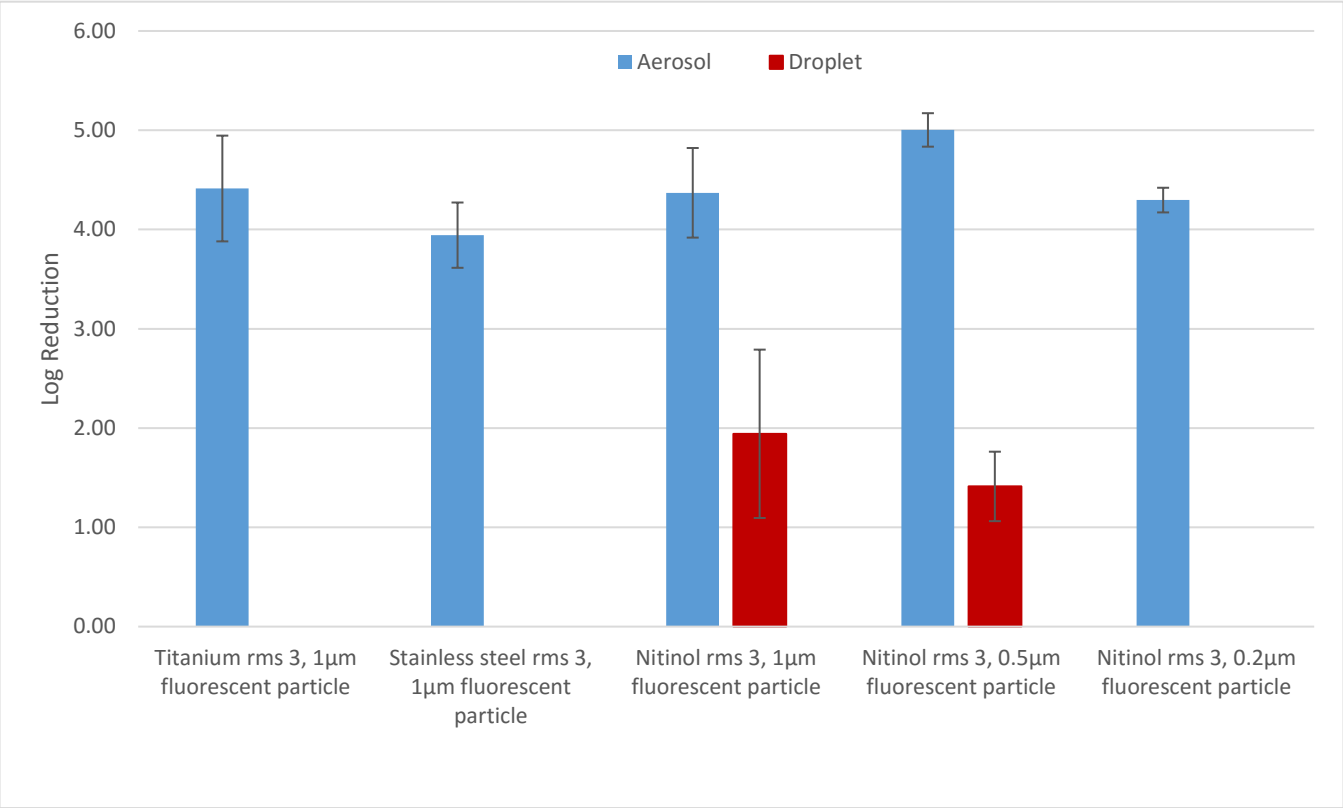
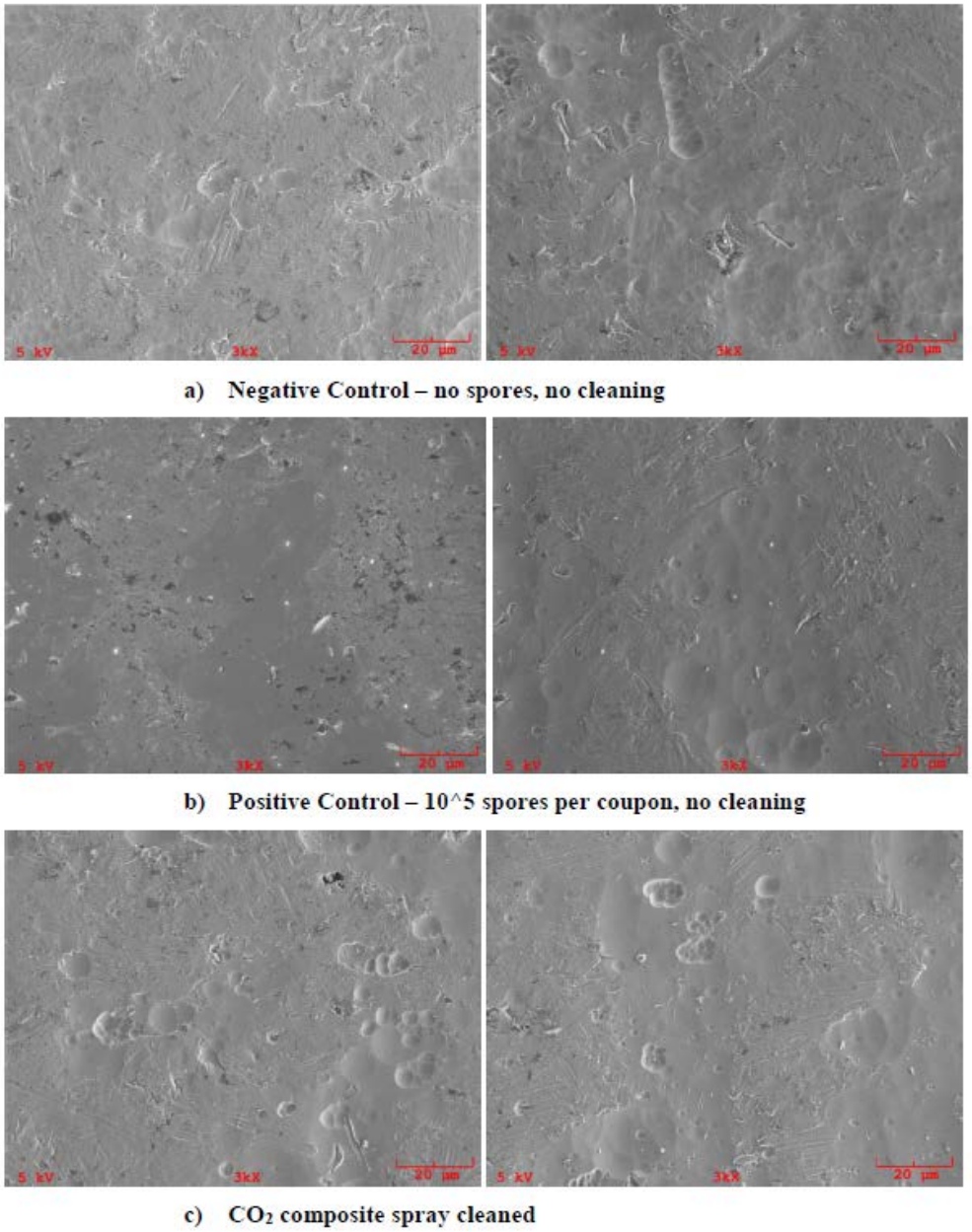


Figure 14: Fluorescent particle analysis, log reduction results summary after cleaning with optimal process parameters on various coupon substrates

V. DISCUSSION

To determine what surface effects, if any, the CO_2 composite spray may have on nitinol, scanning electron micrographs (SEM) images were taken of negative control (no spores, no cleaning) coupons, positive control coupons (~10^5 spore per coupon), and coupons cleaned with the CO_2 composite spray. As seen from Figure 15 below, no significant differences in coupon surface morphology were found between controls and after CO_2 cleaning.



a) Negative Control – no spores, no cleaning

b) Positive Control – 10^5 spores per coupon, no cleaning

c) CO_2 composite spray cleaned

Figure 15: SEM images of Nitinol rms 14 coupon surfaces at 3,000x magnification: a) negative control; b) positive control; and c) after CO_2 composite sprayed cleaned.

VI. Conclusion

Planetary Protection's dry heat microbial reduction process receives a 4-log reduction credit against its starting bioburden. With the CO_2 composite spray system, we were able to attain sterility for aerosol deposited spores and able to match the 4-log reduction for droplet deposited spores. For submicron particles, the CO_2 device is able to clean almost all aerosol deposited particulate contaminants and is able to clean up to a 4-log reduction for droplet deposited particulate contaminants. In short, the CO_2 composite spray device is capable of at least matching the 4-log reduction allowed by the current dry heat microbial reduction process.

VII. Acknowledgements

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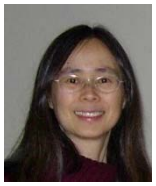
BIOGRAPHY

Nicole Chen is a contamination control engineer at the Jet Propulsion Laboratory (JPL), California Institute of Technology. She received her BA in Physics from Occidental college. She has been involved with the development of the CO_2 composite spray cleaning system and has helped to optimize the system. Nicole is currently acting as support for the Mars 2020 mission.

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.



Shirley Y. Chung is a contamination control engineer at the Jet Propulsion Laboratory, California Institute of Technology. She supported the Mars Science Laboratory project focusing on the Sample Acquisition / Sample Processing and Handling (SA/SPaH) system. Shirley also have extensive planetary protection implementation experience in various Mars flight projects including the Mars Exploration Rovers, Mars Reconnaissance Orbiter, Mars'01, Mars Microprobe DS2, MSP'98 Lander & Payloads, and Mars PathFinder. In addition to flight project work, she was involved in various R&D tasks such as using vapor hydrogen peroxide (VHP) as an alternative method to "sterilize" spacecraft hardware, materials compatibility study with VHP, biological cleaning efficiency studies, and organics cleaning study for the Mars Science Laboratory project.

David Jackson has over 25 years of experience in CO_2 technology development and commercialization. He began his career in the CO_2 development field in 1984 while serving as member of technical staff and group head of precision cleaning and contamination control at Hughes Aircraft Company. While at Hughes he developed first generation CO_2 cleaning technology for producing clean materials for various military and commercial satellite programs. In 1992, he founded the Deflex Corporation, under which he developed and commercialized several CO_2 based cleaning systems. In 2003 Mr. Jackson founded Cleanlogix LLC, which licensed his commercial cleaning and machining products and patent portfolio to Cool Clean Technologies, Inc. He has commercialized over 15 products and holds more than 30 patents. He serves as Senior Vice President and Chief Technology Officer for Cool Clean Technologies, Inc.