Ultrashort Pulse Laser Surface Processing Techniques for Sterilization of Surfaces for Planetary Protection

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Laser-matter interactions

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The Challenge of Bioburden Reduction

Preventing forward contamination of other planetary bodies requires bioburden reduction (sterilization) of spacecraft components to:

- inactivate viable microorganisms
- eliminate cell debris and other potentially confounding biosignatures

Current methods
(mostly Heat Microbial Reduction (HMR)):

- require removing hardware from the assembly room
- are time-consuming and expensive
- are incompatible with sensitive instruments
- do not remove cell debris
- are not effective for eliminating space-hardy, highly resistant microbes such as *Bacillus subtilis* and *Bacillus pumilis*

Viking lander in preparation for HMR

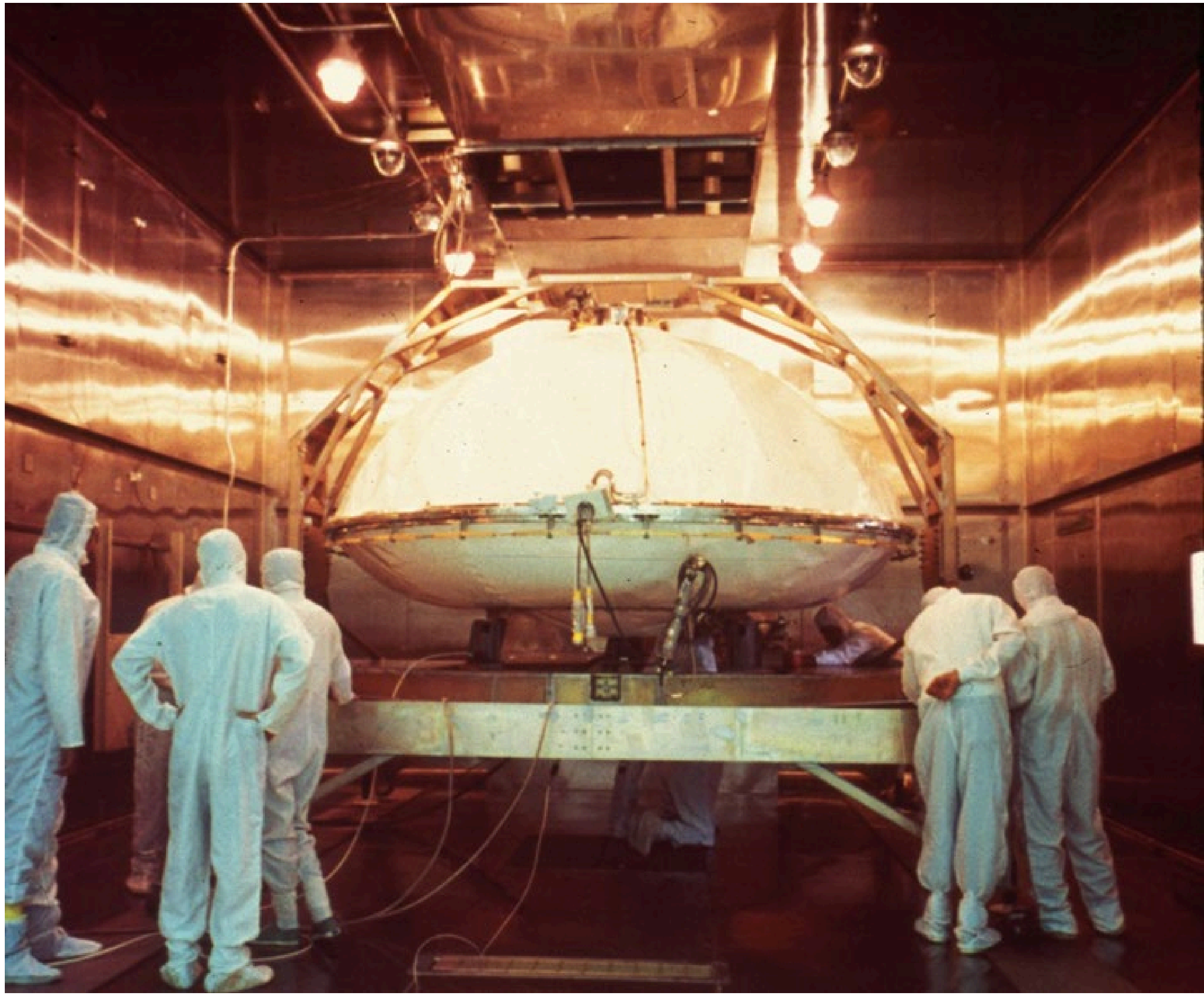


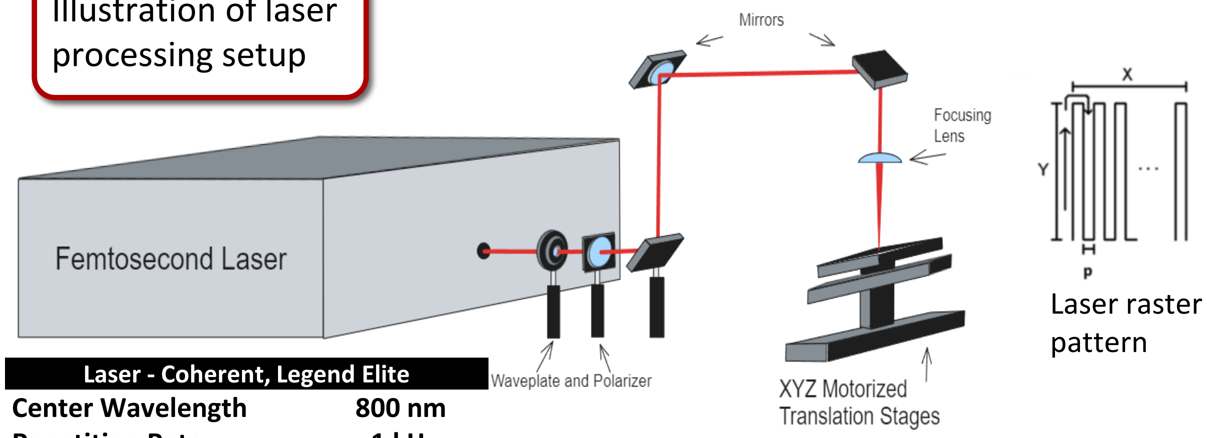
Photo credit: NASA

Why Femtosecond Lasers?

Femtosecond Lasers for Sterilization

- Result in unique and extreme conditions compared to standard optical sterilization sources
- Energy is delivered in an extremely short time, as small as 1 quadrillionth of a second (10^{-15} s), leading to:
  - extreme peak intensities: ~1 x 10^{10} W/cm^2 (UV sterilization lamps ~ 1 x 10^{-6} W/cm^2)
  - extreme photon fluxes: ~1 x 10^{29} photons/(sec*cm^2)
  - large electric fields: ~ 2.74 x 10^7 V/m
  - minimized heat affected zone
- An on-site in situ rapid sterilization technique would substantially reduce the hardware assembly time

Illustration of laser processing setup



Laser - Coherent, Legend Elite	
Center Wavelength	800 nm
Repetition Rate	1 kHz
Pulse Duration	35 fs – 4 ps
Maximum Pulse Energy	10 mJ

Robotic fiber laser cutting system – major advances are being made in laser processing systems for dealing with complex parts

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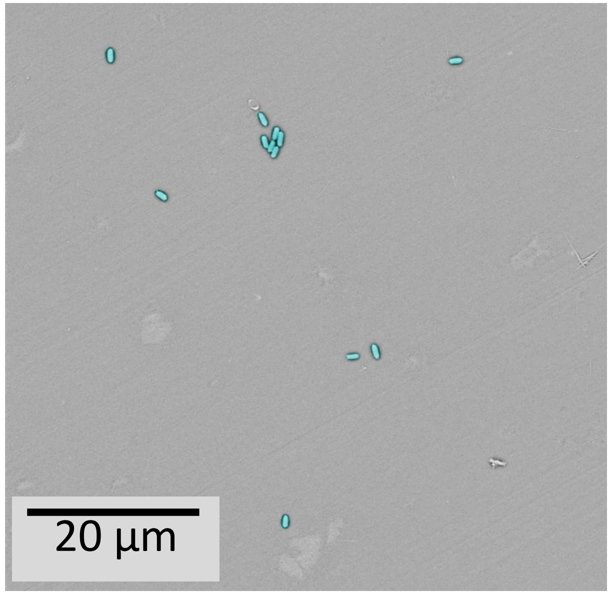
Coupon Preparation and Assaying

Sample inoculation at NASA Ames Research Center (ARC)

- 1x1 cm mirror polished aluminum coupons: a controlled stand-in for spacecraft hardware
- Autoclaved to sterilize

- Inoculated with 2.2×10^{5} CFU of *Bacillus subtilis var. spinzenii* spores
  - Deposit aqueous suspension on coupon
  - Allow to dry in biosafety cabinet
- Shipped to UNL for laser processing



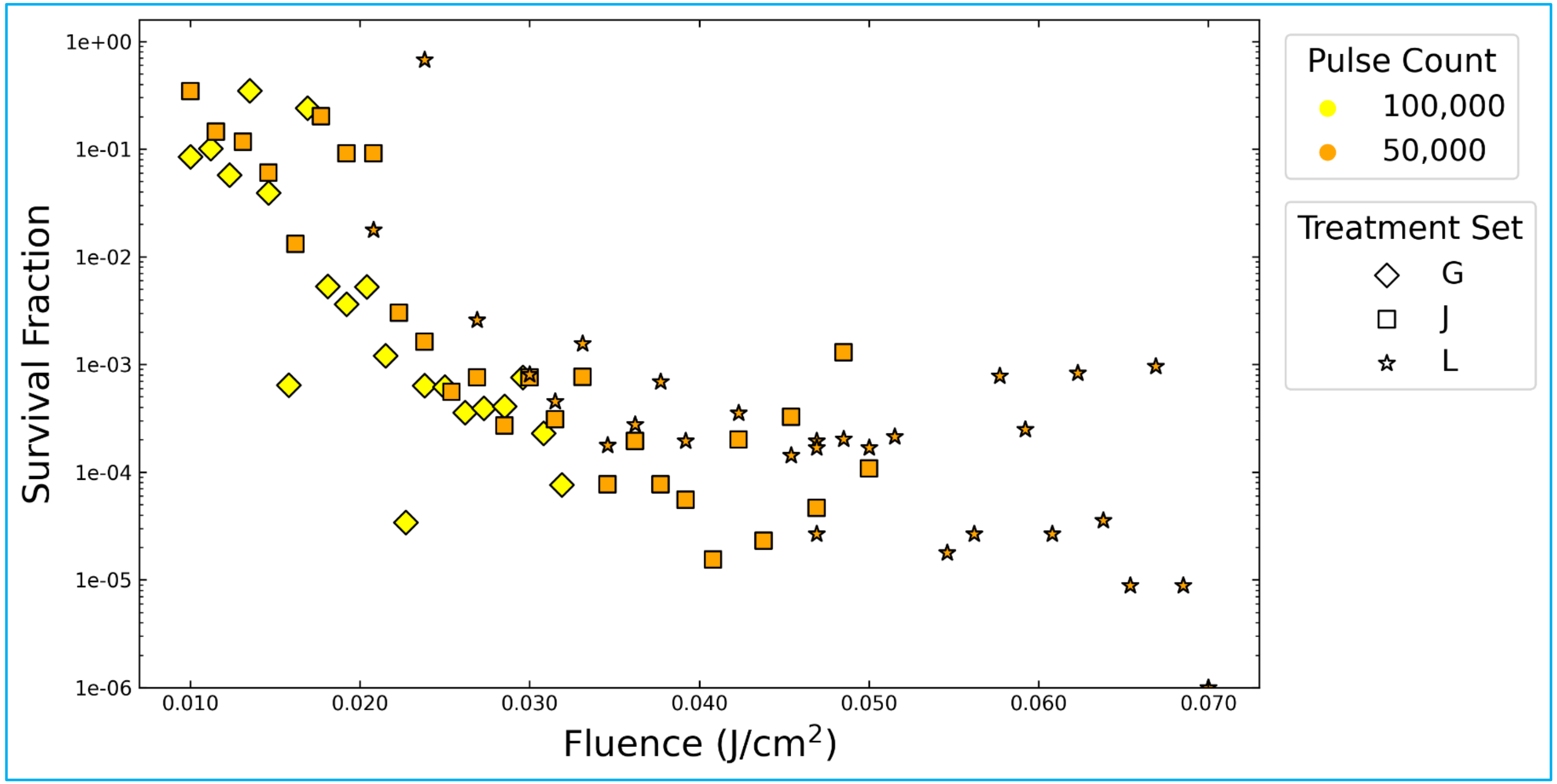
SEM image of spores on a coupon at typical density. False colored for visibility.

Viable spore assay at ARC

- Polyvinyl acetate (PVA) peel method: sterile PVA solution deposited on sample surface, dried, and then peeled off and dissolved into sterile rinse solution of 0.02% Tween-80
- Rinse solution subjected to 10-fold dilutions and plated in molten Tryptic Soy Agar (TSA) in petri dishes
- Bacterial colonies counted and the number of viable spores on each coupon calculated and compared to control to obtain "survival fraction" used in results
- Spore yield from coupons ranges between 80% and 120%

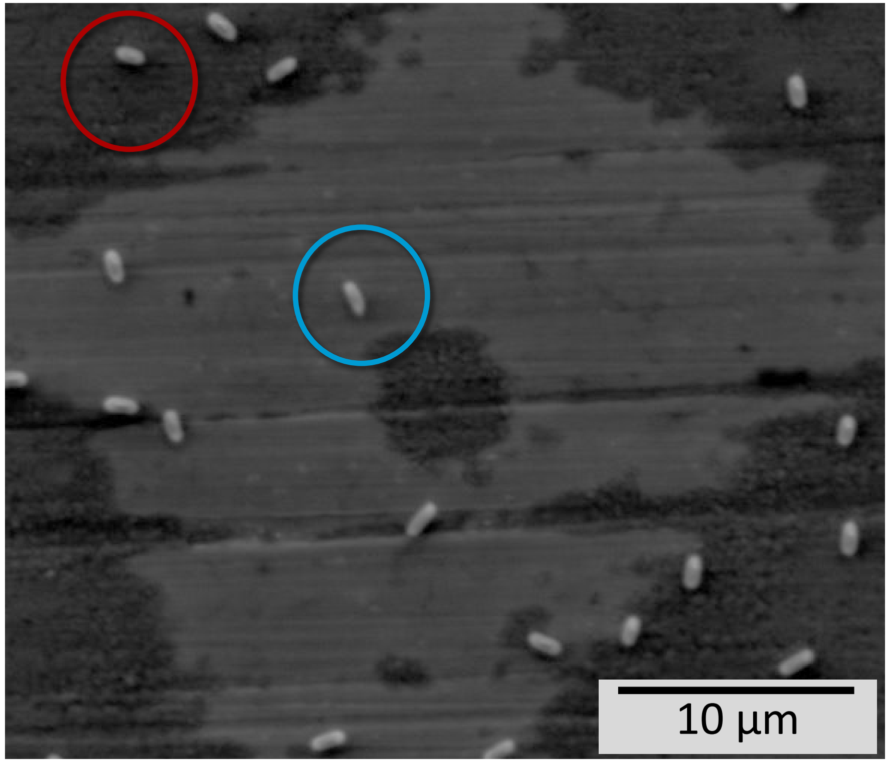
Sterilization Performance

- Wide range of processing parameters are available including fluence, pulse count, and temporal pulse shape.
- Increased sterilization with increased fluence and pulse count, but fluence is more important.
- Have achieved 5-log reduction on some samples and one sample below the detection limit > 6-log reduction
- Higher than 0.04 J/cm^2, non-linear behavior and outliers may be due to shielding from debris from nanoscale surface damage.

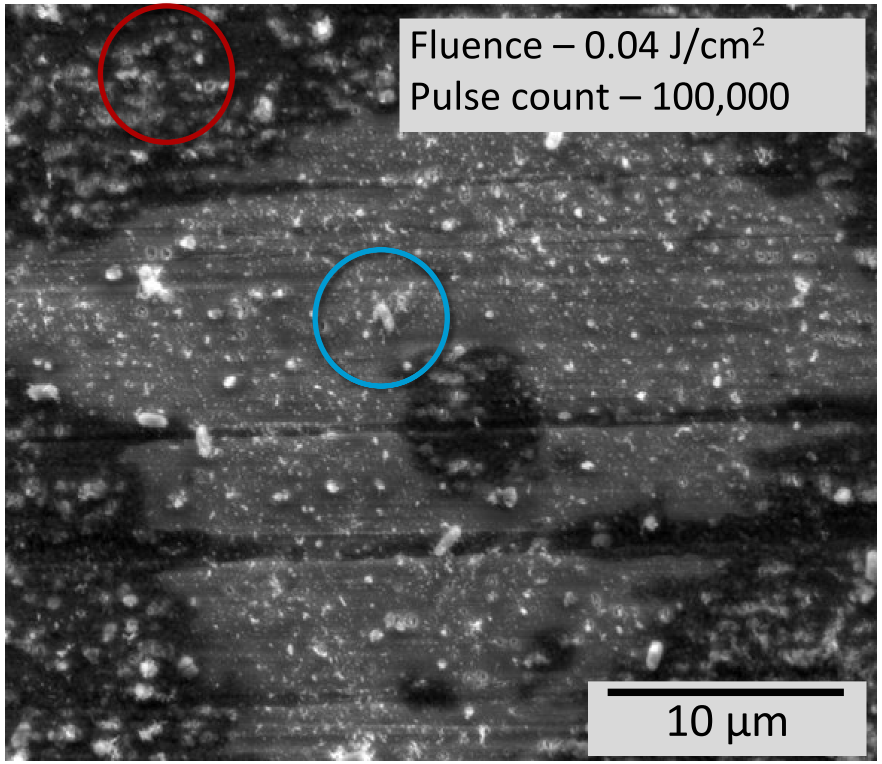


Laser-induced Debris at High Fluence and Spore Removal

- 0.04 J/cm^2 is slightly above the modification threshold, causing minimal nano-scale surface ablation but no structural damage.
- Shockwave from laser irradiation moves spores and may entirely remove some from the sample.



Pre-Processing



Post-Processing

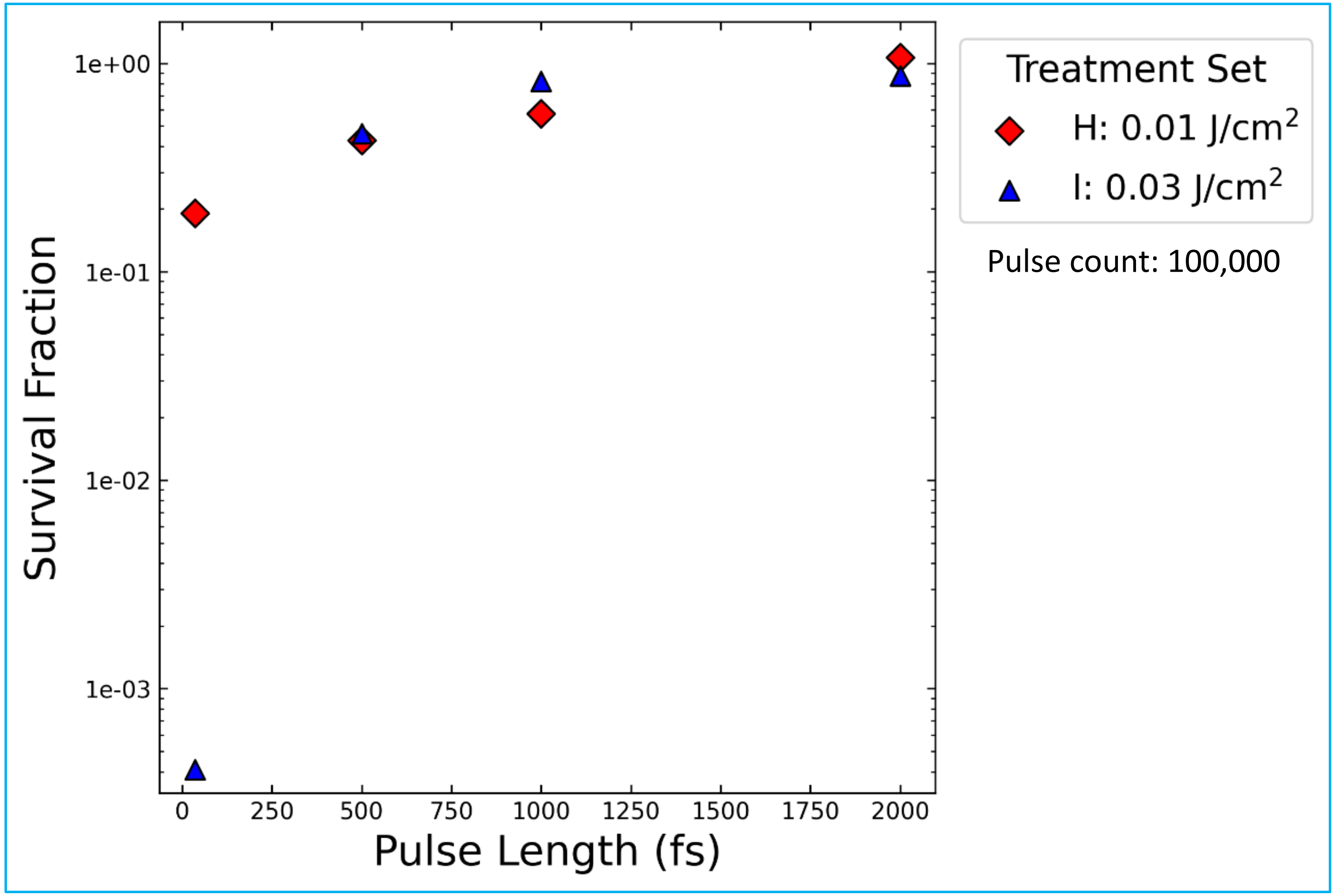
Red circles – spore that has been moved or removed
Blue circles – spore that has remained in place

Shorter Pulse Length is More Effective

Shorter pulses have higher peak intensities and deliver more photons per unit time.

Pulse length	Peak intensity
2 ps	5 GW/cm^2
35 fs	285 GW/cm^2

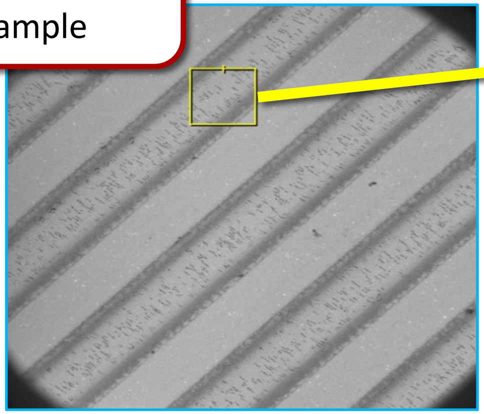
Pulse length, fluence, and pulse count interact to determine sterilization efficiency.



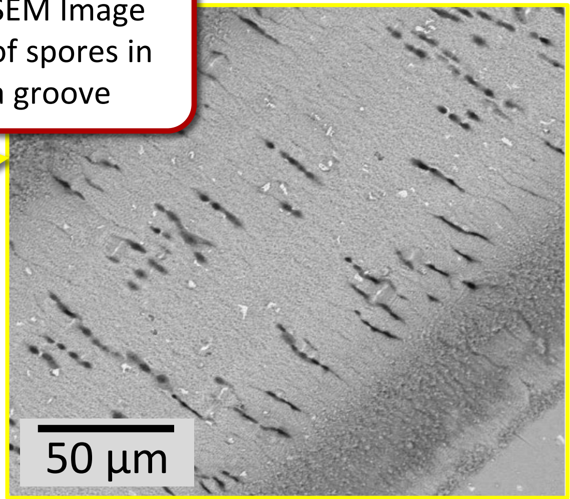
Shorter pulses inactivate more spores.

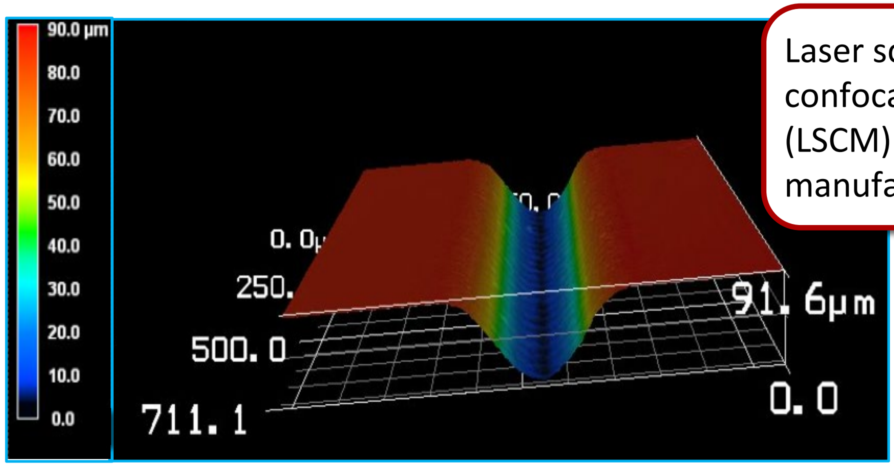
Effectiveness with Non-flat Surfaces

SEM Image of grooved sample



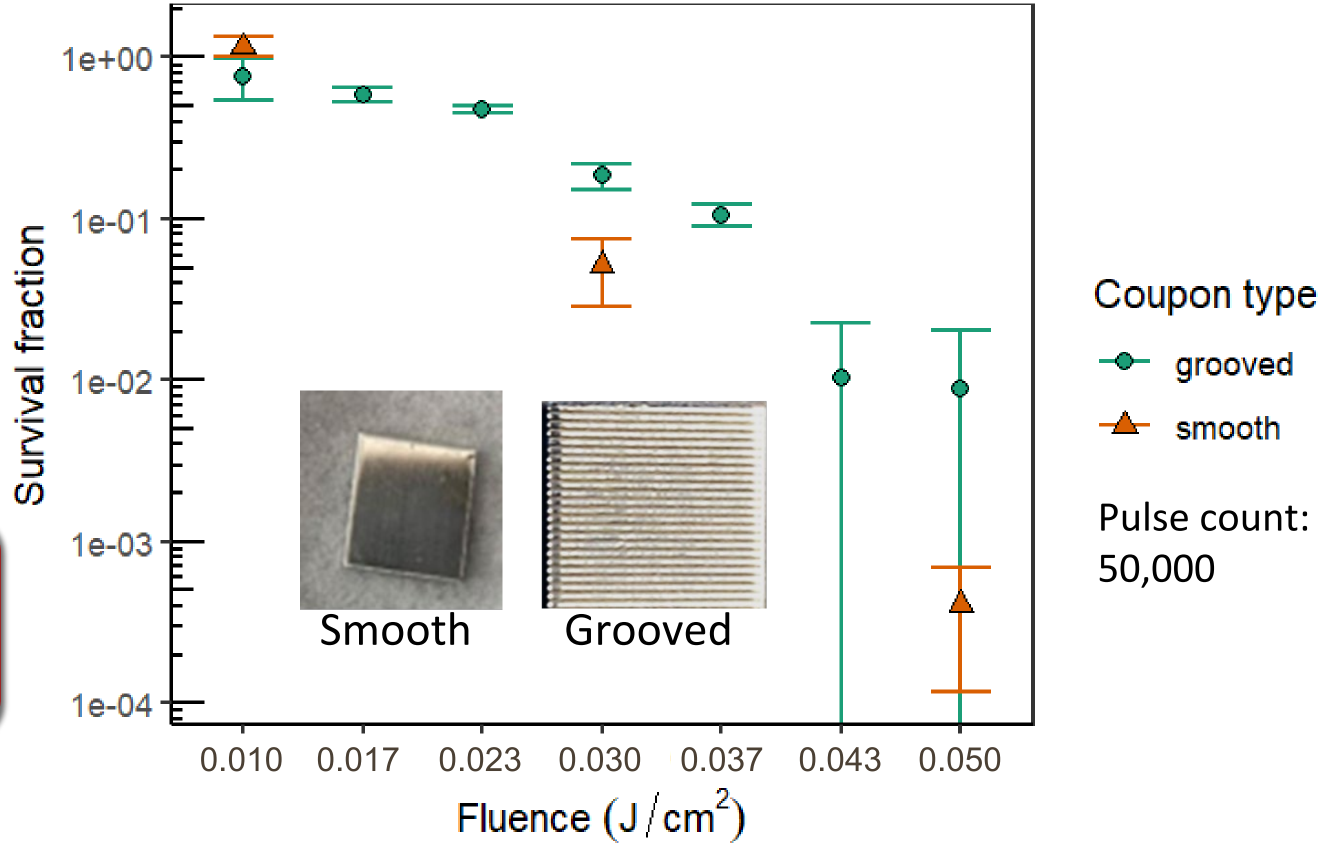
SEM Image of spores in a groove





Laser scanning confocal microscope (LSCM) 3D scan of manufactured groove

Microchannel grooved surfaces were produced using femtosecond laser machining



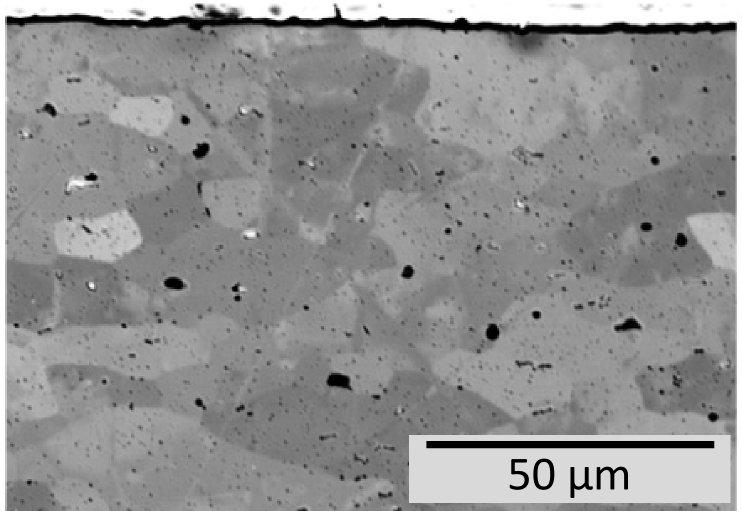
Pulse count: 50,000

Takeaway: An uneven surface provides some protection for spores, but microbial load reduction is still meaningful

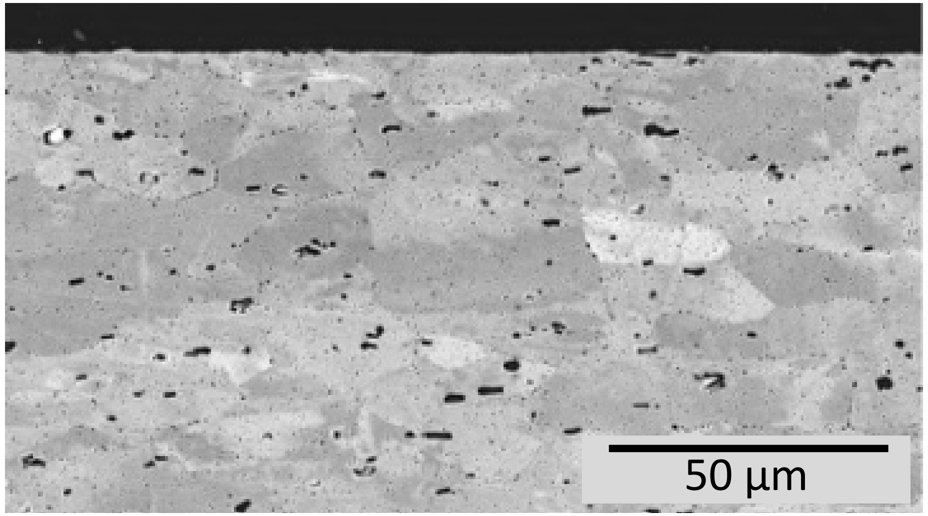
Results

Damage-free Subsurface

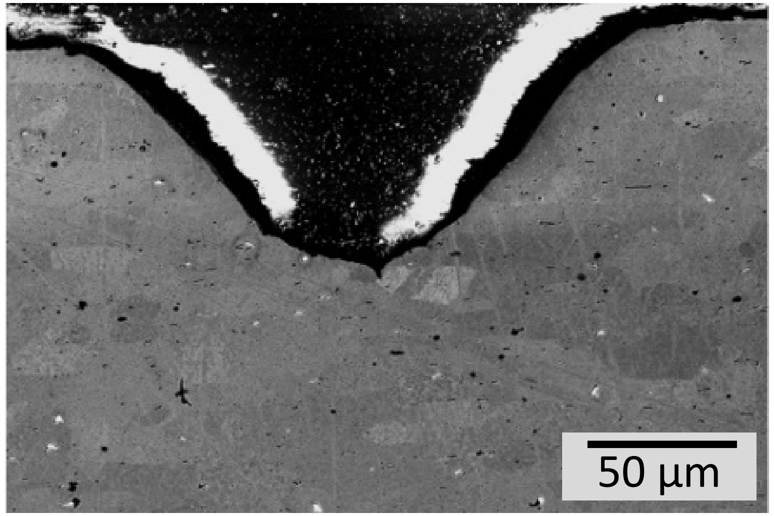
Cross-sectional materials analysis completed at the Jet Propulsion Lab (JPL)



Untreated



Fluence: 0.05 J/cm^2
Pulse count: 50,000
Survival fraction: 1.49x10^{-4}

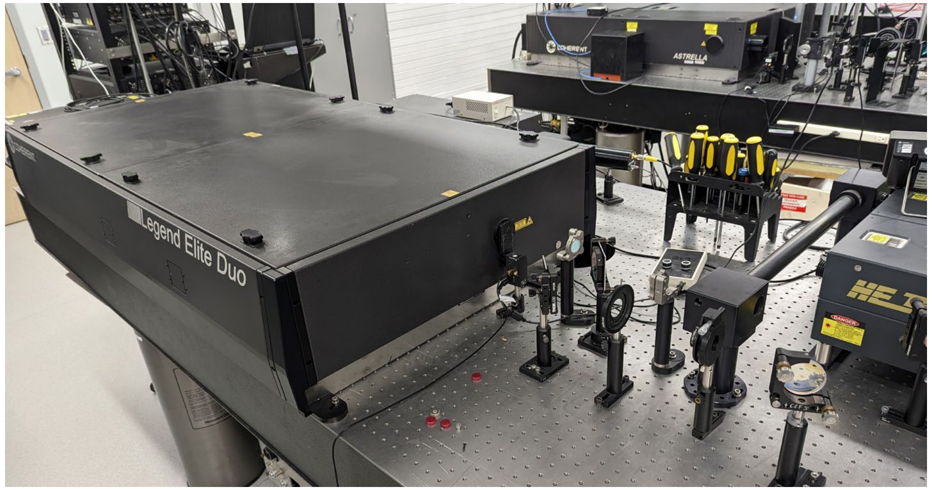


Laser-grooved surface (grooved surface shown on previous slide)

No subsurface damage for sterilized sample or laser-grooved surface – minimized heat affected zone with femtosecond pulses

The Next Generation of Ultrashort Pulse Lasers for High-Speed Sterilization

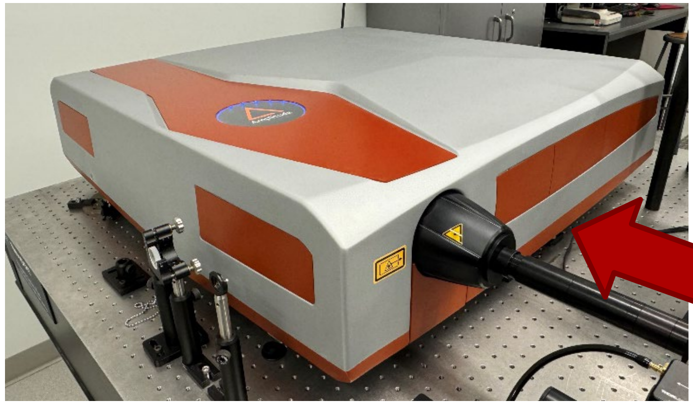
Old Laser System

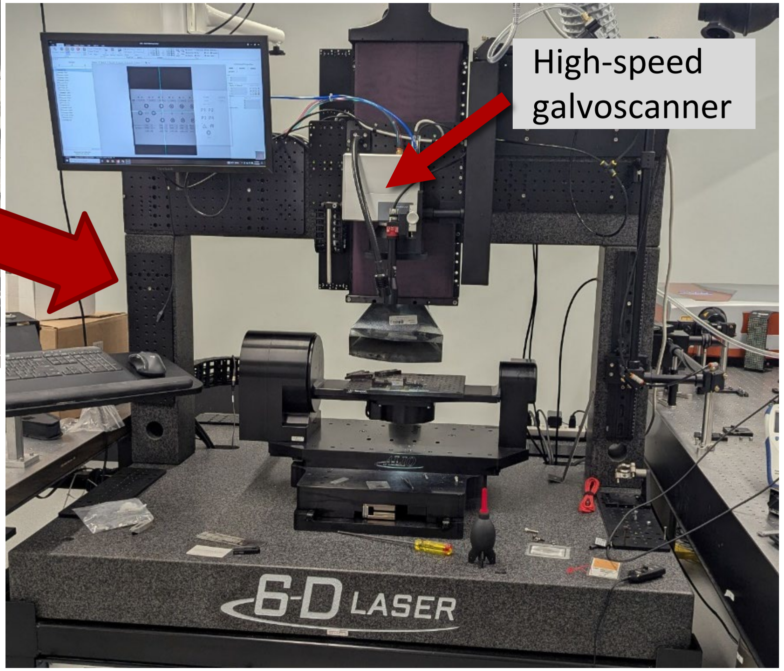


- Coherent, Legend Elite Duo
- Pulse Energy: 10 mJ (operated at 6 mJ used)
- Repetition rate: 1 kHz
- **Avg. Power: 10 W (operated at 6 W)**
- Wavelength: 800 nm
- Pulse length: 35 fs – 5 ps

New Laser System

(DURIP-funded, UNL had first in the US, delivered in Oct. 2023)





- Amplitude, Tangor 300
- Pulse Energy: 3 mJ
- Repetition rate: 1 – 40 MHz
- **Avg. Power: 300 W**
- Wavelength: 1030 nm
- Pulse length: 500 fs – 10 ps

5 axis stage system and galvoscanner for high-speed processing with 3D capabilities

Higher average power and ability to control repetition rate is expected to lead to increases in processing rate by as much as two orders of magnitude (targeting greater than 100 in^2/hr for current ROSES project)

Thank you

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