

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

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*Laser-matter interactions*

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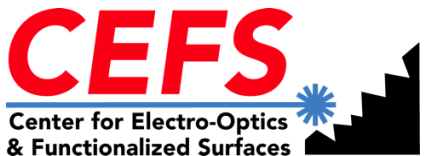
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**Preventing forward contamination** of other planetary bodies requires bioburden reduction (sterilization) of spacecraft components to:

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

## Current methods

(mostly Heat Microbial Reduction):

- 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

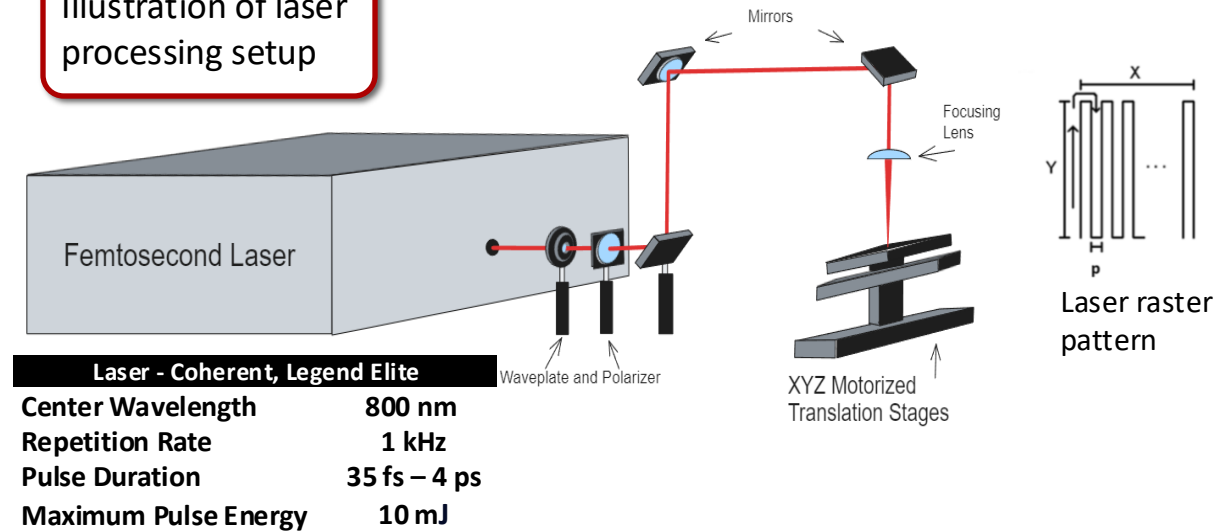


Photo credit: NASA

## Femtosecond Lasers for Sterilization

- ❖ An on-site in situ rapid sterilization technique would substantially reduce the hardware assembly time
- ❖ Energy is delivered in an extremely short time, as small as 1 quadrillionth of a second ( $10^{-15}$  s), leading to:
  - extreme intensities:  $\sim 1 \times 10^{10}$  W/cm<sup>2</sup>
  - extreme photon fluxes:  $\sim 1 \times 10^{29}$  photons/(sec\*cm<sup>2</sup>)
  - minimized heat affected zone
- ❖ Result in unique and extreme conditions compared to standard optical sterilization sources

Illustration of laser processing setup



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



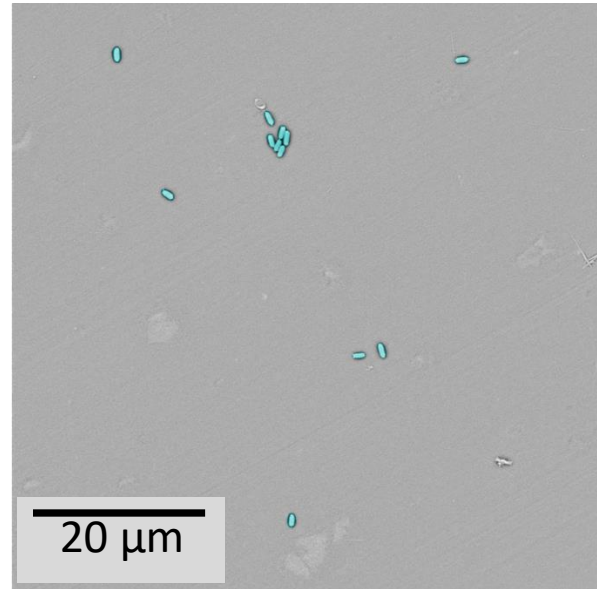
<https://stock.adobe.com/images/Fibre-laser-robotic-remote-cutting-system> by Andrei Armiagov

## Sample inoculation at ARC

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



- Inoculated with  $2.2 \times 10^5$  CFU of *Bacillus subtilis* var. *spizzenii* spores
  - Deposit aqueous suspension on coupon
  - Allow to dry in biosafety cabinet
- Shipped to UNL for laser processing

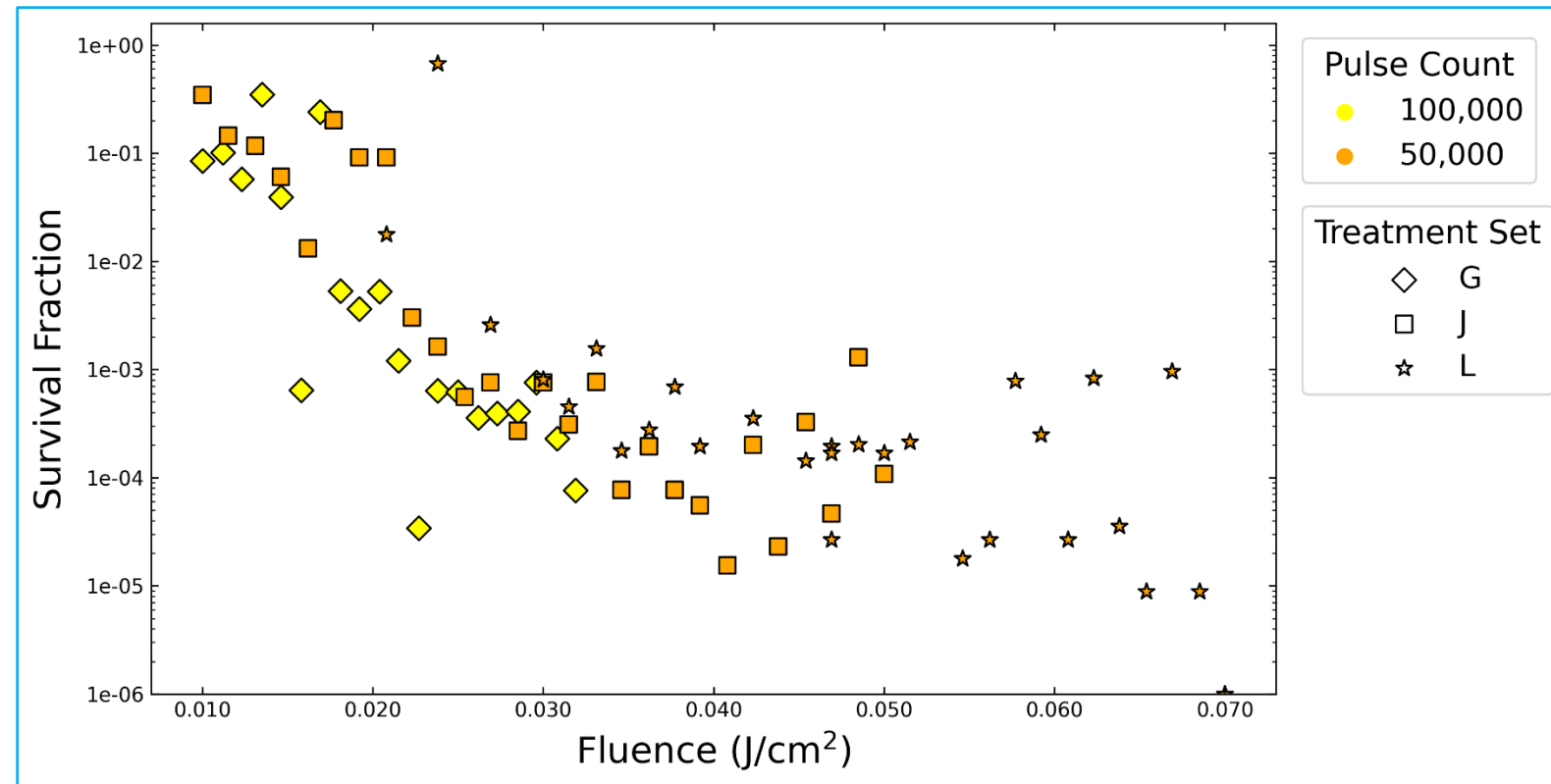


SEM image of spores on a coupon at typical density. False colored for visibility.

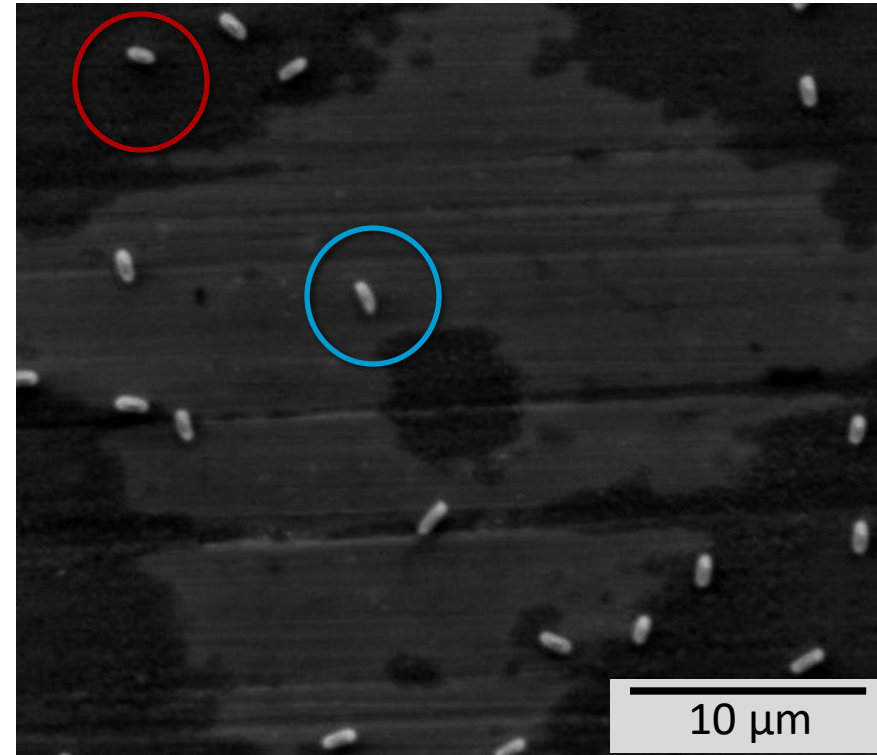
## Viable spore assay at ARC

- 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%

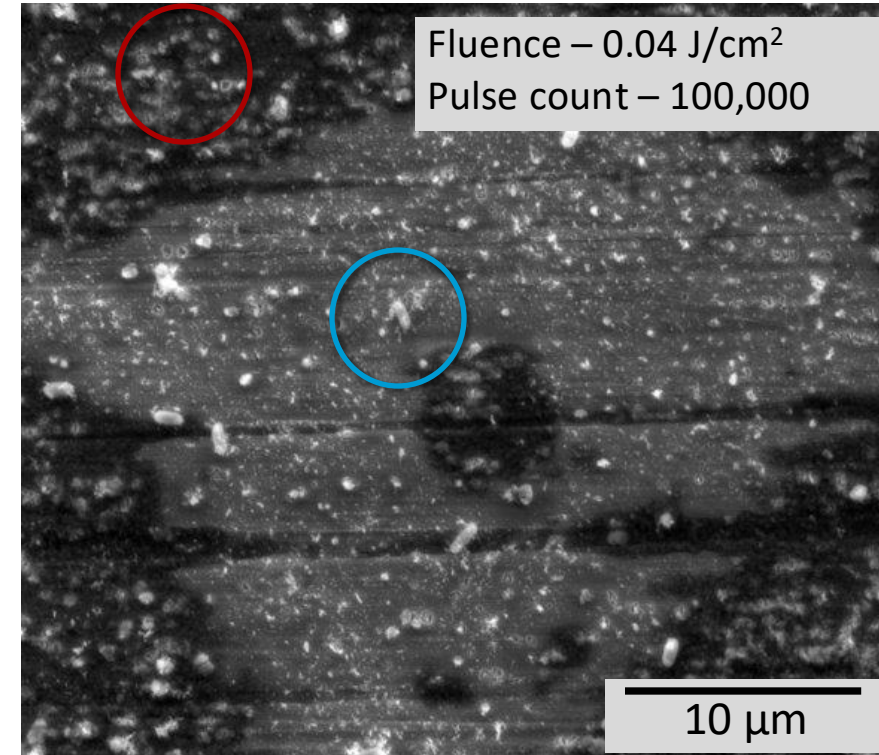
- Wide range of processing parameters are available including fluence, pulse count, and temporal pulse shape.
- Increased sterilization with increased fluence and pulse count, but fluence is more important.
- Have achieved 5-log reduction on some samples and one sample below the detection limit > 6-log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



- $0.04 \text{ J/cm}^2$  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

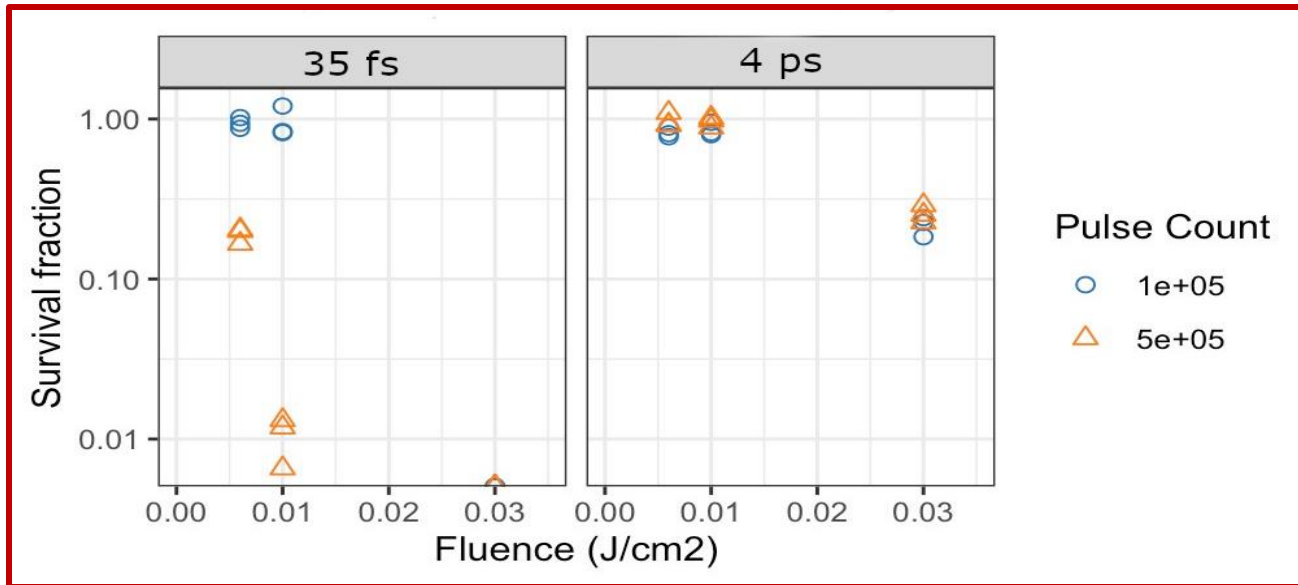


Post-Processing

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

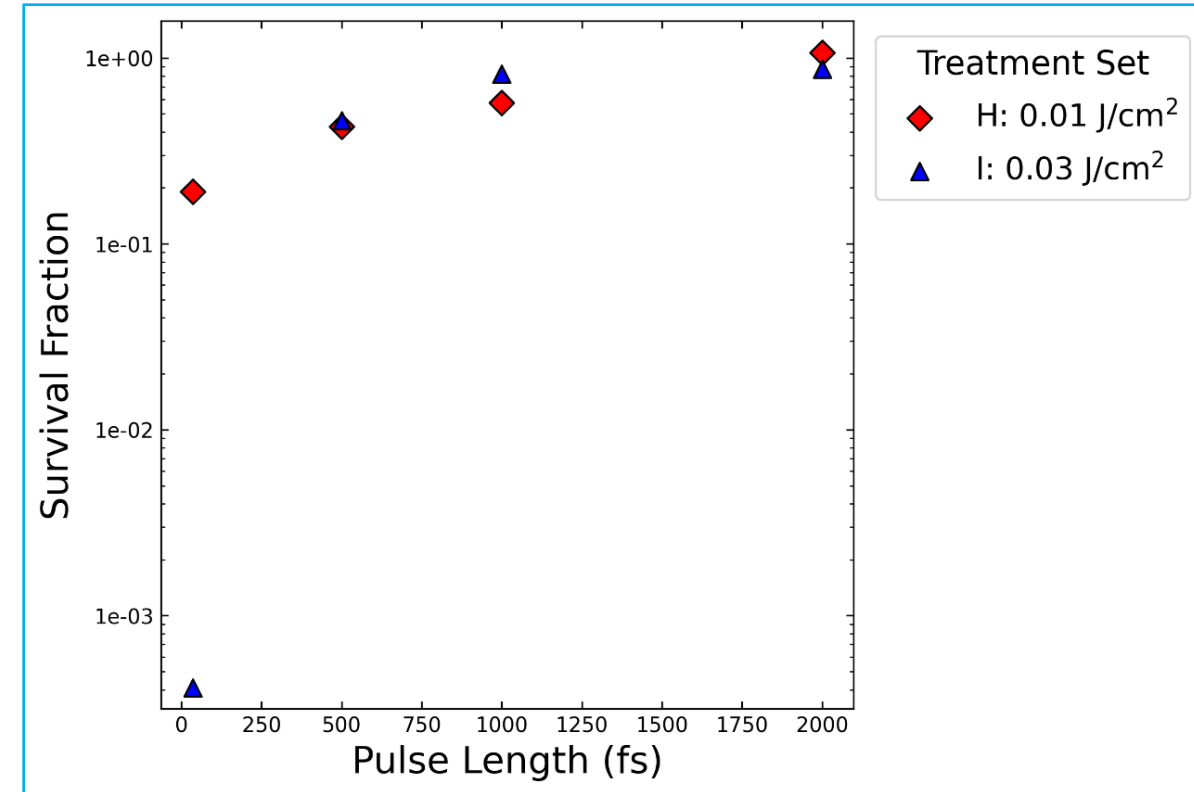
Shorter pulses have higher intensities and deliver more photons per unit time.

| <u>Pulse length</u> | <u>Peak intensity</u>  |
|---------------------|------------------------|
| 4 ps                | 2.5 GW/cm <sup>2</sup> |
| 35 fs               | 285 GW/cm <sup>2</sup> |



35 fs is more effective at sterilization than 4 ps.

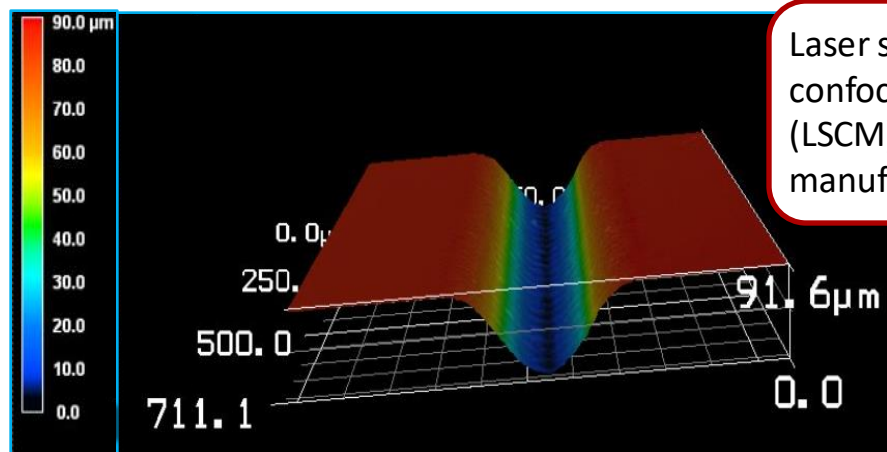
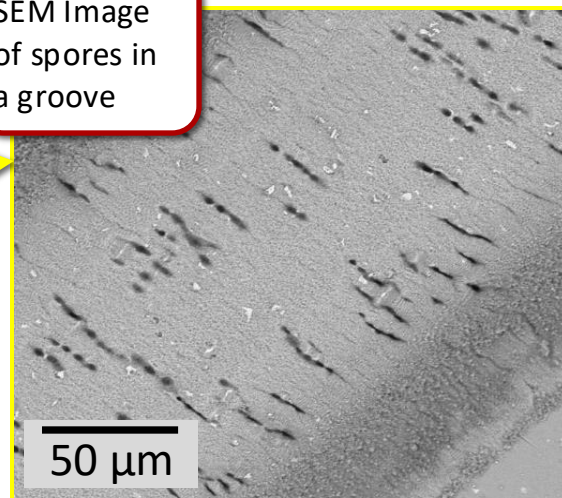
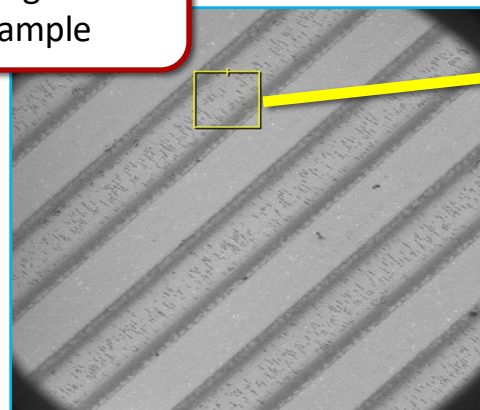
Pulse length, fluence, and pulse count interact to determine sterilization efficiency.



Shorter pulses inactivate more spores.

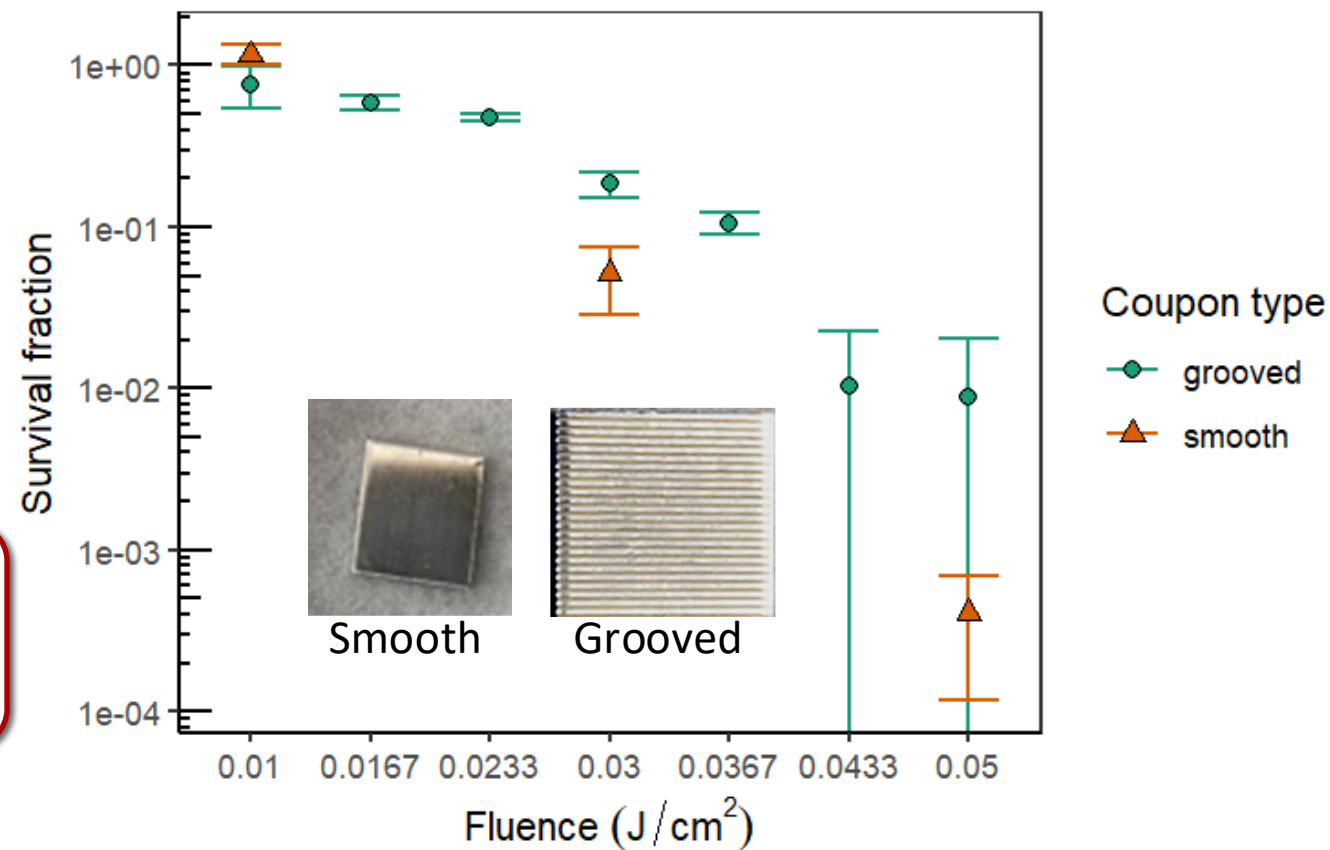
SEM Image of grooved sample

SEM Image of spores in a groove

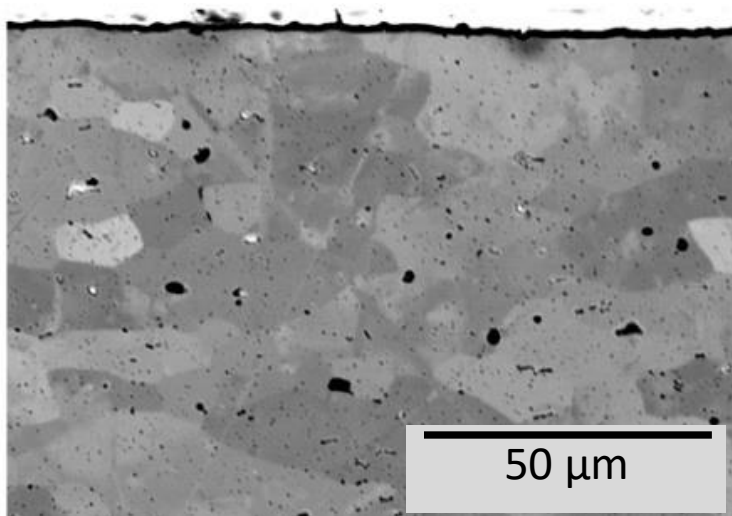


Laser scanning confocal microscope (LSCM) 3D scan of manufactured groove

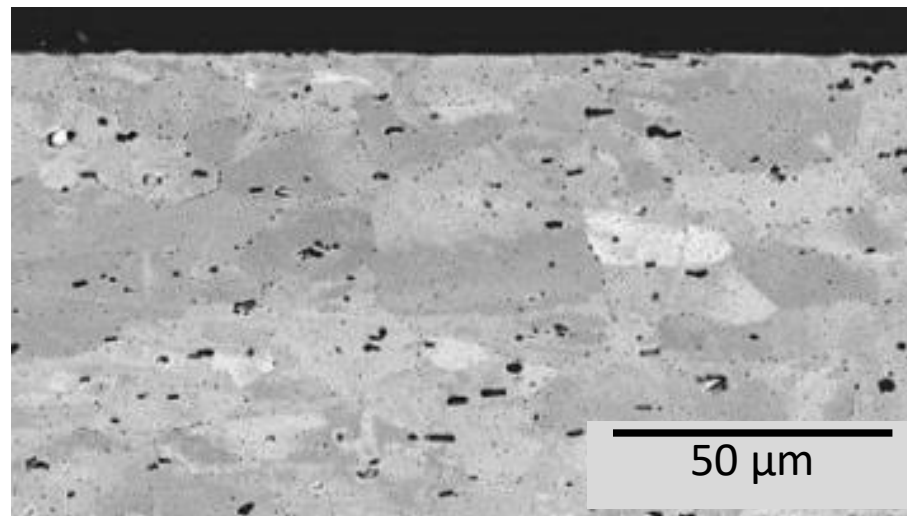
Microchannel grooved surfaces were produced using femtosecond laser machining



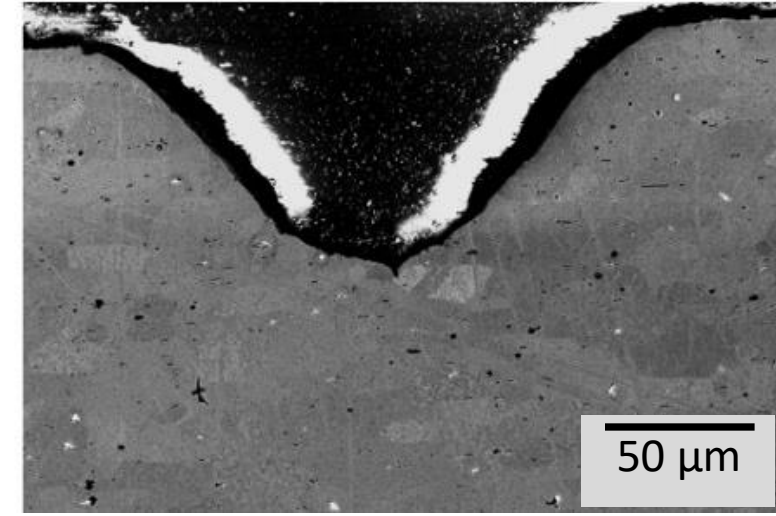
**Takeaway:** An uneven surface provides some protection for spores, but microbial load reduction is still meaningful



Untreated



Fluence:  $0.05 \text{ J/cm}^2$   
Pulse count: 50,000  
Survival fraction:  $1.49 \times 10^{-4}$



Laser-grooved surface

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

## Old Laser System



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

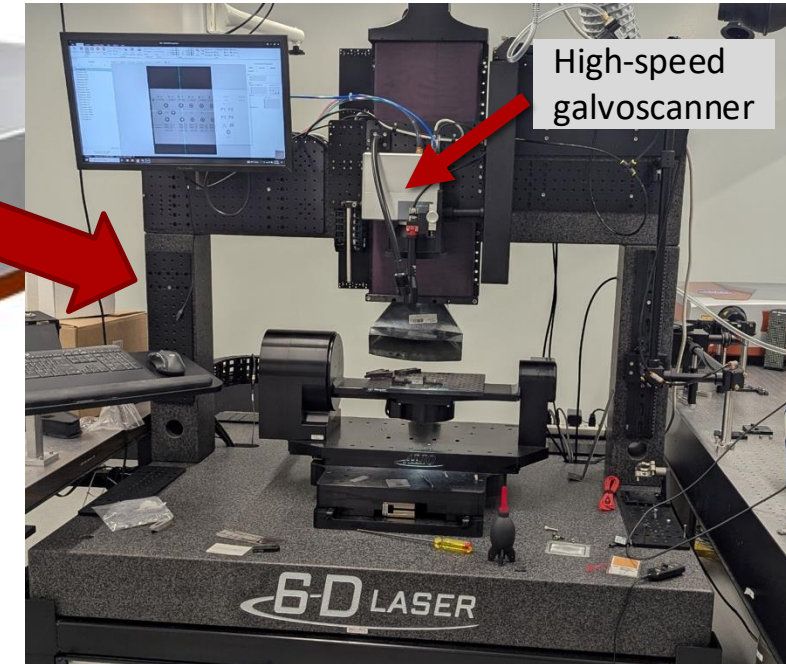
**Higher average power and ability to control repetition rate is expected to lead to increases in processing rate by as much as two orders of magnitude (targeting greater than 100 in<sup>2</sup>/hr for current ROSES project)**

## 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 galvoscanter for high-speed processing with 3D capabilities

# Questions?

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