Laser-Induced Breakdown Spectroscopy Emission Enhancement from Bacteria on a Silver Thin Film

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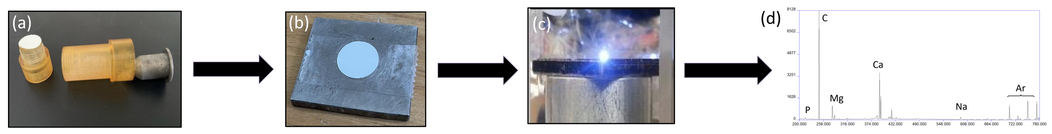
Motivation

There is high demand in clinical settings for real-time identification of bacterial pathogens in biological specimens. We investigate the use of laser-induced breakdown spectroscopy (LIBS) for this purpose. The goal of this research is to enhance our LIBS emission signal in order to detect smaller concentrations of bacteria and better discriminate between bacteria species.

We want to improve on our previous work of enhancement with silver microparticles by using a different deposition technique that consistently provides a uniform distribution of silver.

Introduction

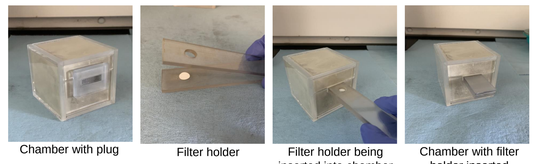
LIBS is a spectrochemical technique used to rapidly determine elemental composition. A laser pulse is focused onto a target material which absorbs the laser's energy, converts it to heat, and vapourizes the target. The vapour then begins to absorb the laser energy to create a plasma. As the plasma cools, photons are emitted and collected by our spectrometer to create a time-resolved optical emission spectrum. Bacteria is **(a) centrifuged through a metal cone** onto a nitrocellulose filter. The filter is **(b) mounted onto a steel piece** and **(c) ablated in an argon environment**. Finally, **(d) a broadband atomic emission spectrum is acquired**.



The emission spectrum acts as a "fingerprint" of the bacteria's elemental composition. The ratios between intensities of the elemental emission lines differs between bacteria species, and this is what allows us to differentiate between them.
Silver is deposited on the ablation surface (the filter) in order to increase the intensity of our emission spectrum. As a metal, silver has many free electrons that it can donate to the LIBS plasma, thus increasing the temperature of the plasma.

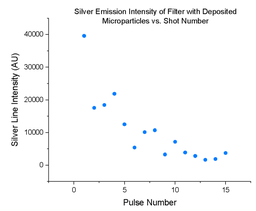
Previous Work with Silver Microparticles[1,2]

Method:
A plexiglass chamber was constructed to deposit silver microparticles. The chamber was filled with silver then agitated to disperse the microparticles. The filter was inserted on a filter holder to be coated with a trace amount of microparticles as they settled under the influence of gravity.

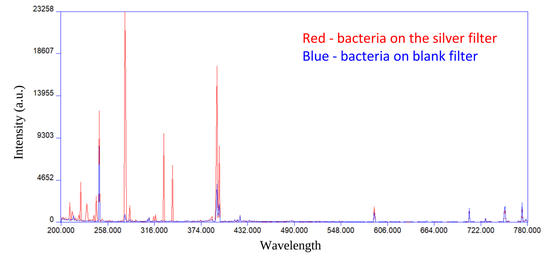


Problem:
20-30 spectra are acquired from each filter, but the plasma shockwave created by the pulses displaces the silver, causing a decrease in the amount of ablated silver after each pulse. This reduces spectral enhancement.

How do we address this?



Results:
Enhanced emission was observed in the LIBS emission spectra when silver microparticles were deposited on the filter. The enhancement ratio ranged from 1-10 for all of the species and elements, with an average enhancement ratio of 4.3.



Bacteria Species	C	P	Mg	Ca	Na
E. coli	1.3	4.6	3.9	5.4	3.9
M. smegmatis	1.2	1.7	2.7	8.4	6.7
E. cloacae	1.2	4.4	6.9	2.2	1.3

Enhancement ratios of elemental emission line intensity with silver to without silver

Results of Pulsed Laser Deposition: Enhancement of Bacteria Spectra

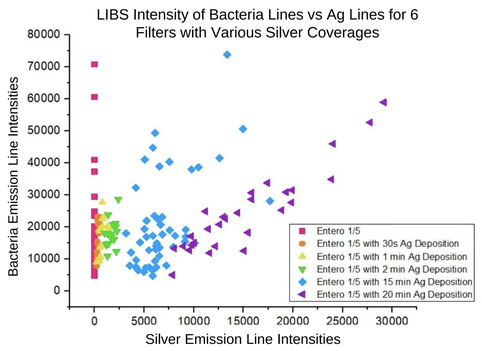
Enhancement of Total Spectral Power

Filters were coated with different amounts of silver by using different sputtering times. The filters were removed from the vacuum chamber, bacteria were deposited, and LIBS was performed on the depositions.

20-30 single-shot LIBS spectra were acquired from each filter. Similar to the uncoated filters (shown in red), these spectra exhibit variations in spectral intensity due to the stochastic nature of LIBS.

Each filter exhibited a linear relationship between bacterial line intensity and silver line intensity. More intense plasmas caused every elemental line in the spectrum to increase in intensity.

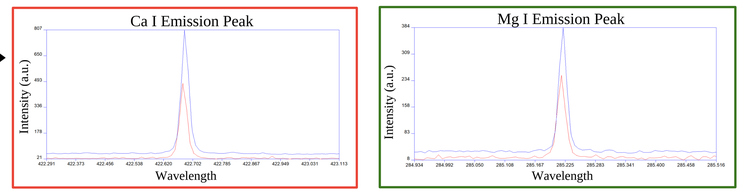
The bacterial LIBS intensity increased for very long deposition times (>15 min). However, this enhancement was minimal relative to the variation in the data.

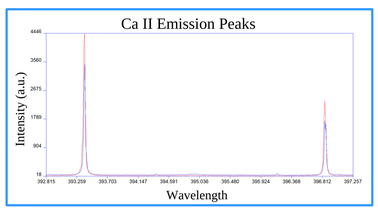


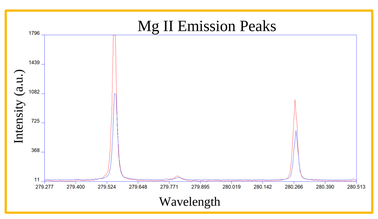
Enhancement of Individual Peaks

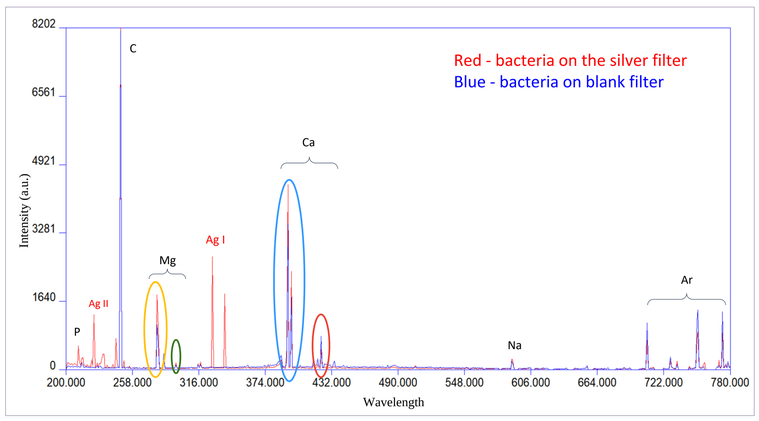
Ca and Mg neutral emission is **lower** on silver filters than on the blank filters

Ca and Mg singly-ionized emission is **enhanced** on silver filters compared to on blank filters





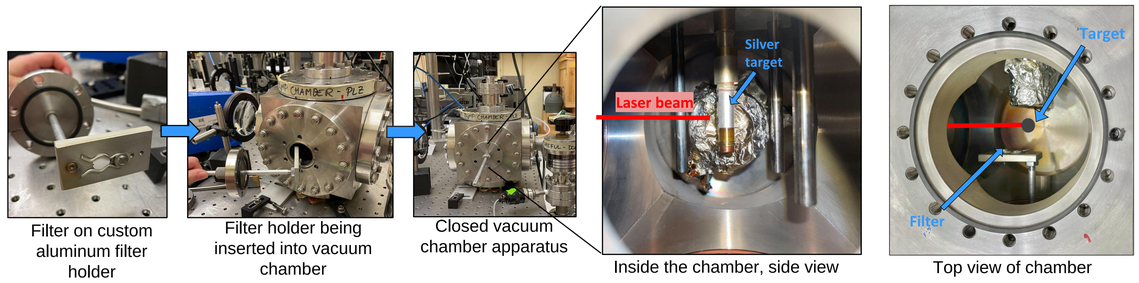




Result:
An increase in the temperature of the plasma is inferred from the increase in the fraction of singly-ionized emission.

Pulsed Laser Deposition

A 1064 nm pulsed laser (60 mJ per pulse) is focused onto a rotating silver target inside a vacuum chamber (~10 mTorr). Ablated silver atoms are sputtered onto a filter substrate placed in proximity to the target.

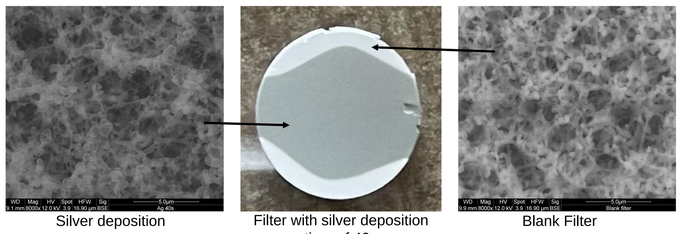


Filter on custom aluminum filter holder | Filter holder being inserted into vacuum chamber | Closed vacuum chamber apparatus | Inside the chamber, side view | Top view of chamber

Results of Pulsed Laser Deposition: Uniformity of Silver Film

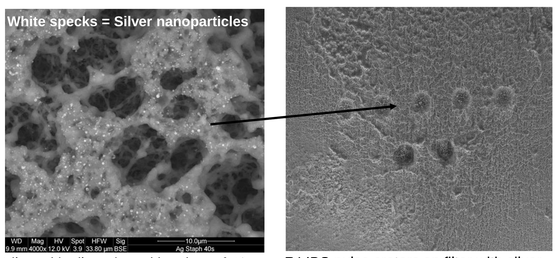
Scanning Electron Microscope (SEM) images:

The deposition is so uniform and thin it appears the same as a blank filter, although it is easily visible to the naked eye.



Silver deposition | Filter with silver deposition time of 40s | Blank Filter

In images of areas around the craters of the LIBS pulses, the silver film was observed to be disturbed, creating nanoparticles.



Filter with silver deposition time of 40s | 7 LIBS pulse craters on filter with silver deposition time of 40s

This ability to produce nanoparticles could be useful for our future investigations of nanoparticle-enhanced LIBS (NELIBS).

LIBS Measurements of Silver:

The silver line intensity exhibits variation characteristic of all of our measurements; however, there is no longer a decrease in intensity with subsequent pulses. The sputtered thin film is more robust than the silver microparticle deposition.

problem is solved ✓

Conclusions and Future Work

Conclusions:
- A uniform layer of silver was sputtered and shown to be more robust than the microparticle deposition
- Less bacterial enhancement was observed compared to when silver microparticles were used
- Emission intensity increased for singly-ionized Mg and Ca lines while the intensity decreased for neutral lines, indicating an increase in the temperature of the plasma

Future work:
- Deposit nanoparticles on filters to enhance the bacterial signal (NELIBS) by:
  - Performing laser sputtering in air to create nanoparticles
  - Producing nanoparticles around laser pulse craters as seen previously
  - Performing laser sputtering in water to create nanoparticles in suspension

Acknowledgements

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References

[1] Jeremy C. Marvin, Emma J. Blanchette, Sydney C. Sleiman, Haiqa Arain, Emily A. Tracey, Steven J. Rehse, Silver Microparticle Enhanced Laser-Induced Breakdown Spectroscopy, Applied Spectroscopy, May 2022.
[2] Jeremy C. Marvin, "*Signal optimization and enhancement of laser-induced breakdown spectroscopy for discrimination of bacterial organisms*", M.S. thesis, Dept. of Physics, University of Windsor, Windsor, 2020.