Bacterial Mounting and Concentration Techniques to Translate Laser-Induced Breakdown Spectroscopy into a Clinical Setting

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Introduction

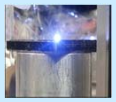
Motivation:
There is high demand for real-time identification of bacterial pathogens in biological specimens, and laser-induced breakdown spectroscopy (LIBS) is a promising technique for accomplishing this.

What we do:

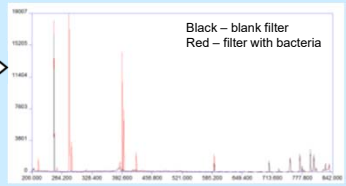
Trypticase soy agar (TSA) is used to culture bacteria.

Colonies are removed and placed in 1.5 mL of deionized water.

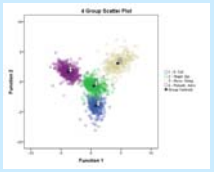
Sample is vortexed and 30 µL is deposited on a 0.45 µm nitrocellulose filter paper.



Filter paper is mounted on steel piece and ablated with laser.



Upon laser ablation, a spectrum of the sample is obtained, revealing the sample's elemental composition.



Bacteria can be discriminated based on elemental composition.

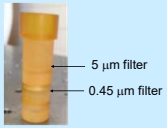
Experimental parameters:

- Nd:YAG laser
  - 1064 nm
  - 10 Hz repetition rate
  - 8 ns pulse duration
  - 10 mJ/pulse incident on target
- Échelle spectrometer with spectral coverage from 200 – 800 nm
- 2 µs gate delay
- 20 µs gate window
- 20 SCFH argon environment

Separation of a Contaminant from a Bacterial Suspension

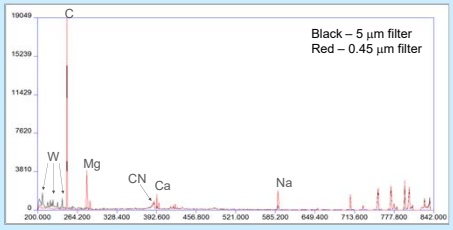
In a clinical setting, the biological sample to be tested will likely consist of unwanted cells that would need to be separated from the bacteria prior to testing with LIBS. A quick method of separating a contaminant from a bacterial suspension using centrifugation and filter media with different pore sizes was investigated.

An insert for a centrifuge tube was designed by a previous student as a tool for depositing bacterial suspensions on filter media.



An *E. coli* suspension with tungsten powder (12 µm APS) as the contaminant was deposited in the insert with the 5 µm filter paper on top and the 0.45 µm filter paper below it.

The tungsten powder was caught by the 5 µm filter while ~ 80% of the bacteria passed through it and settled onto the 0.45 µm filter.



Conclusion:
The use of this centrifuge tube insert with filter media of different pore sizes was effective at separating a contaminant from bacteria.

Future work:
Repeat this experiment using yeast cells as the contaminant to more closely simulate the cells that would be present in a clinical sample (such as red blood cells), then proceed to testing with clinical samples such as blood.

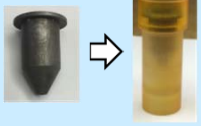
Bacterial Concentration to Improve Limit of Detection

Number of bacterial cells present in clinical samples:

- < 100 CFU/mL in blood[1]
- 0 – 200 CFU in nasal swab[2]

Our current bacterial LOD: ~ 50 000 CFU per laser ablation event

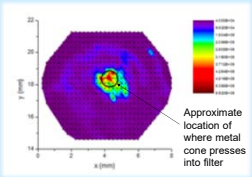
How can we lower our LOD?
We designed a metal cone to fit inside an insert for a centrifuge tube.



Bacteria is forced onto a smaller area of the filter paper, increasing the number of cells ablated per laser shot.

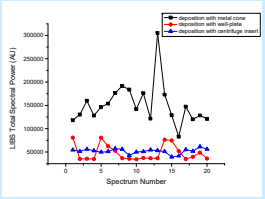
The metal cone was used to deposit an *E. coli* suspension on a filter paper and 570 LIBS spectra were acquired across it to obtain an intensity map of the bacterial deposition on the filter.

Each point on the map corresponds to a laser shot, and the color indicates the LIBS bacterial intensity, with purple indicating no LIBS bacterial signal, and red indicating the region with the strongest LIBS bacterial signal.



Approximate location of where metal cone presses into filter

The same *E. coli* suspension was deposited on filter papers using different deposition methods, and 20 spectra were acquired across each filter.



The total spectral power is given by the sum of the area-under-the-curve intensities of the elemental emission lines present in a bacterial spectrum.

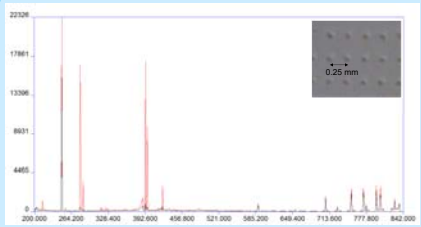
Conclusion:
The use of the metal cone for deposition on the filter paper as opposed to other deposition methods results in an increased LIBS bacterial signal.

Future work:
Acquire LIBS data for different concentrations of bacteria to compute a LOD for bacteria that is deposited on a filter paper with this metal cone.

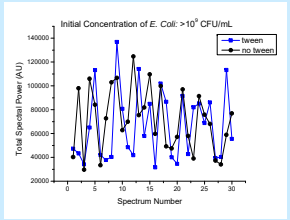
Prevention of Bacterial Clumping

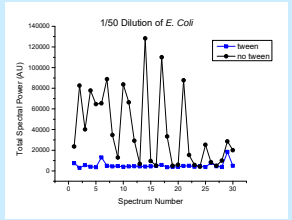
Bacterial cells clump together resulting in non-uniform laser ablation, preventing determination of a LOD. This clumping is particularly problematic at low concentrations. When deposited on the filter paper, we observe some LIBS spectra with high bacterial signal (red) and some with little to no bacterial signal (black) located 0.25 mm apart.

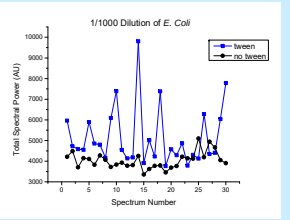
How can we prevent this clumping?
Tween 20 is a detergent used to solubilize cells. Its effectiveness at preventing the bacteria from clumping was investigated.

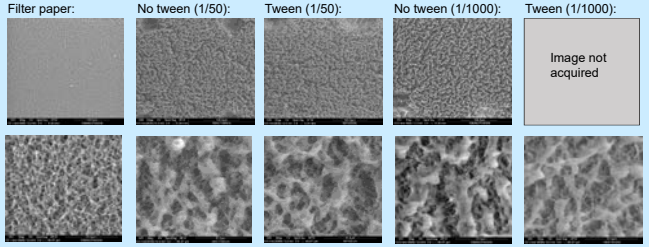


Two sets of dilutions were prepared from the same initial suspension of *E. coli*. One set was treated with a 0.1% concentration of Tween while the other set was not treated with Tween as a control. Each dilution was deposited on nitrocellulose filter papers and 30 LIBS spectra were acquired across the filters. Scanning electron images of the ablation regions were obtained after LIBS testing.







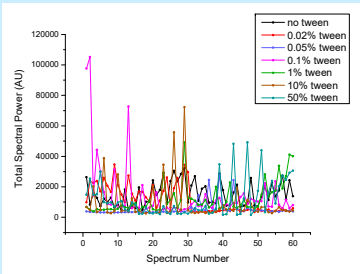


Comparison of the last two graphs indicate that the total spectral power of the 1/50 dilution of *E. Coli* without Tween is ~10-20 times greater than that of the 1/1000 dilution. However, the corresponding scanning electron images show no such difference in the amount of bacteria deposited. The same can be said of the depositions with and without Tween for the 1/50 dilution of *E. Coli*.

The reason for this disagreement between the graphs and the scanning electron images is not known at this time.

Various concentrations of Tween were added to *E. coli* suspensions of the same concentration. Each suspension was deposited on a different filter medium and 60 LIBS spectra were acquired.

There does not seem to be a specific concentration of Tween that yields a relatively constant LIBS bacterial signal around the average LIBS signal of the clumped bacteria.



Conclusion:
Tween was not effective at preventing *E. coli* from clumping in the experiments that were performed.

Future Work:

- Repeat these experiments with a different species of bacteria. Treatment with Tween may not be effective on *E. coli* due to its gram-negative and/or rod-like structure.
- Culturing of the bacteria in liquid media rather than on solid media may prevent clumping. The bacteria grow dispersed in liquid media, which should significantly lessen clumping and/or make the use of Tween more effective. Suspensions prepared in this way would also more closely resemble clinical specimens.

References:
[1] *Rapid microbial sample preparation from blood using a novel concentration device*. A. Boardman *et al.*, PLoS ONE, 2015.
[2] *Nasal screening for MRSA: Different swabs – different results!* P. Warnke *et al.*, PLoS ONE, 2014.