Concentration of Cells and Elimination of Extraneous Background Signals in Laser–Induced Breakdown Spectroscopy to Identify, Differentiate and Detect Bacteria

Jeremy Marvin, Sydney Sleiman, Emma Blanchette, Allie Paulick, Steven J. Rehse
University of Windsor, Windsor, ON, Canada

Introduction

There is an urgent need to develop faster ways to identify pathogenic bacteria. Our aim is to develop a real-time point-of-care medical diagnostic technology utilizing Laser - Induced Breakdown Spectroscopy (LIBS). Our procedure consists of common materials and equipment that could be easily implemented in a clinical setting to rapidly identify bacteria based on their elemental composition, so that more targeted treatment can begin as soon as possible.

Current Method of Analyzing Bacteria

...if Bacteria is present and swabbed from a surface

Clean metal cone, metal plate, and test tube insert with bleach water solution and rinse with DI water.

Deposit 100 µL of bacteria suspended in DI water onto metal plate and heat for 2 min and 20 s.

Deposit 10 µL water onto swab tip and swab metal plate.

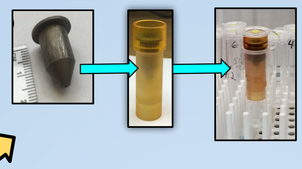
Place swab in centrifuge tube with 1 mL of water and vortex tube for 15 s.

The swab was tested for traces of contamination and did not contribute to background emission.

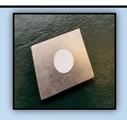
Place a 0.45 µm filter on the base piece. Connect the base. Place the cone in the insert.

Cleaning the metal plate had a significant impact but the method of cleaning yielded similar results.

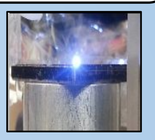
Deposit the 1 mL of sample from vortexed tube into cone, place insert into clean centrifuge tube. Centrifuge insert at 5000 rpm, 2500 g's for 5 min.



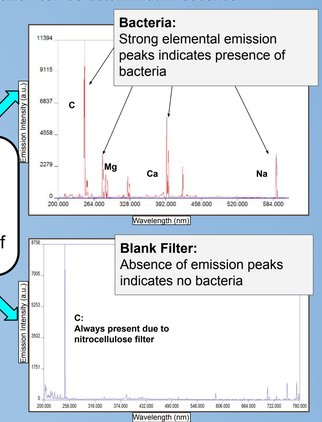
Remove insert(s) from centrifuge, then remove filter(s) from insert. Mount 0.45 µm filter on metal plate with double - sided tape.



Place filter in an argon environment and ablate using a pulsed 1064 nm Nd: YAG laser.

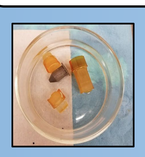


Échelle diffraction grating spectrometer is used to disperse the emission spectrum which determines the elemental composition of the sample.



...if Bacteria is present in a liquid suspension

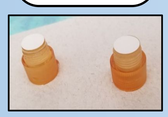
Clean metal cone and test tube insert with bleach water solution and rinse with DI water.



Pipette 100 µL of liquid contaminant containing bacteria suspension into a centrifuge tube.

Add 1mL water to the tube and vortex for 15 s.

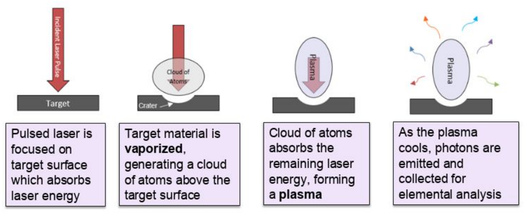
Mount a 5 µm filter and a 0.45 µm filter on two separate insert base pieces.



Connect both base pieces to the insert, with the 5 µm filter on top. Place the cone in the insert.

Laser-Induced Breakdown Spectroscopy (LIBS)

LIBS is an **elemental analysis technique**



Pulsed laser is focused on target surface which absorbs laser energy

Target material is **vaporized**, generating a cloud of atoms above the target surface

Cloud of atoms absorbs the remaining laser energy, forming a **plasma**

As the plasma cools, photons are emitted and collected for elemental analysis

Measured Atomic Emission Lines In Bacterial LIBS Spectra

Element	Emission Line Wavelength (nm)
Carbon (**C**)	247.856
Phosphorus (**P**)	213.618, 214.914, 253.398, 253.560, 255.326, 255.491
Magnesium (**Mg**)	277.983, 279.079, 279.553, 279.806, 280.271, 285.213
Calcium (**Ca**)	317.933, 393.366, 396.847, 422.673
Sodium (**Na**)	588.995, 589.593

LIBS Advantages

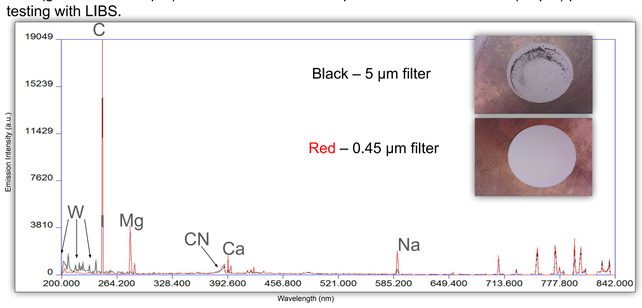
- Can be done on solids, liquids, gases and bacteria
- Requires only µg of sample
- Elemental composition can be determined in seconds

Scope of the Work

Laser ablation of the filter medium and other elemental contaminants yields a non - zero background signal when a control experiment is performed in the absence of any bacterial cells. The purpose of this research was to optimize the bacterial sample preparation protocol by identifying the source of this background signal and introducing new cleaning procedures that reduce this background signal. Following this preparation procedure, the ability to discriminate and/or identify low titers of different types of pathogenic bacteria using the centrifuge insert / cone bacterial concentration technique is being investigated.

Separation of a Contaminant from a Bacterial Suspension - Dual Centrifugation

In a clinical setting, the biological fluid specimen to be tested will likely consist of unwanted cells (greater than 10 µm) that would need to be separated from the bacteria (~1 µm) prior to testing with LIBS.

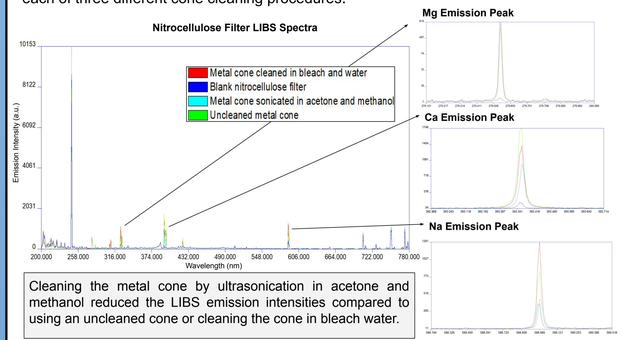


An *E. coli* suspension with **tungsten powder** (12 µm APS) as the contaminant was deposited in the insert with the 5 µm filter paper on top and the 0.45 µm filter paper below it. The tungsten powder was caught by the 5 µm filter while ~ 90% of the bacteria passed through it and settled onto the 0.45 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 based on its size.

The Effect of Cleaning the Metal Cone

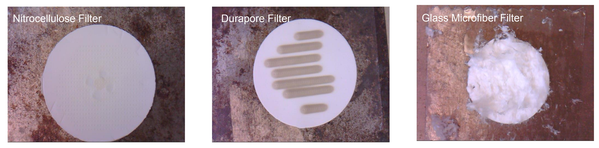
20 LIBS filter spectra were acquired when water (no bacteria) was centrifuged through the cone to compare the elemental intensities of carbon, sodium, magnesium and calcium for each of three different cone cleaning procedures.



Cleaning the metal cone by ultrasonication in acetone and methanol reduced the LIBS emission intensities compared to using an uncleaned cone or cleaning the cone in bleach water.

Conclusion:
Cleaning the metal cone with an appropriate procedure yielded a significant reduction in the emission peak intensities in the absence of bacteria.

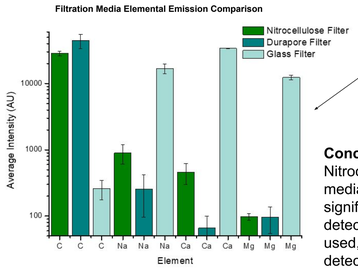
The Effect of the Filtration Media on the LIBS Emission Spectra



- Uniform Surface
- Ca, Mg and Na present
- Significant C emission observed
- Current medium for our LIBS testing procedure
- Nitrocellulose membrane

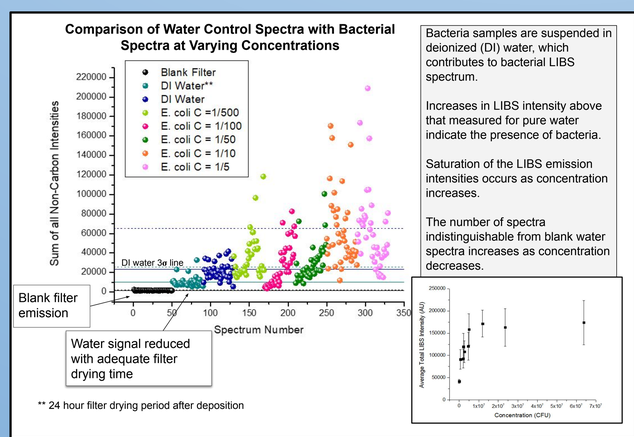
- Uniform Surface
- Reduced Ca, Mg, and Na observed
- **C emission increased**
- Significant scorching after laser ablation
- (PVDF) Hydrophilic Polyvinylidene Fluoride

- Significant increase in Ca, Mg, and Na observed
- Significant reduction in C emission
- **Destruction of testing surface after laser ablation**



Comparison of elemental emission intensities of four elements of interest: carbon, sodium, calcium, and magnesium

Conclusion:
Nitrocellulose is the best of the three media tested, but the presence of a significant carbon peak limits the detector amplification which can be used, which limits our current limit of detection (LOD).



Bacteria samples are suspended in deionized (DI) water, which contributes to bacterial LIBS spectrum.

Increases in LIBS intensity above that measured for pure water indicate the presence of bacteria.

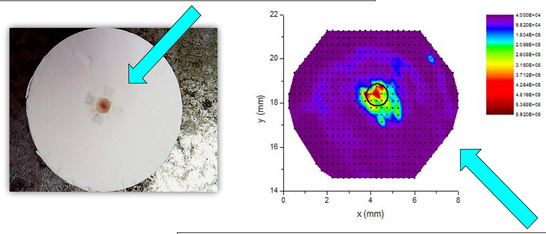
Saturation of the LIBS emission intensities occurs as concentration increases.

The number of spectra indistinguishable from blank water spectra increases as concentration decreases.

** 24 hour filter drying period after deposition

Improving the Limit of Detection

Bacteria concentrated to a smaller area on the filter paper to increase the number of cells ablated per laser shot

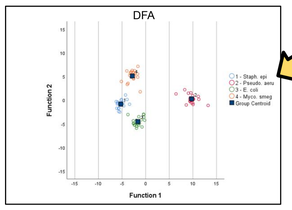


Intensity map of the bacterial deposition on the filter. Purple indicates regions with no LIBS bacterial signal, and red indicates regions with the strongest LIBS bacterial signal

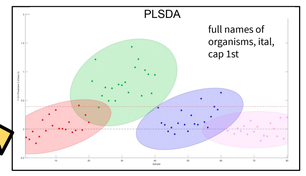
Conclusion:
The use of the metal cone for deposition on the filter paper as opposed to other deposition methods results in an increased LIBS bacterial signal and improves our LOD by an order of magnitude.

Chemometrics: DFA and PLSDA

Because of the similarity in bacterial LIBS spectra, computerized chemometric signal-processing algorithms must be used to distinguish LIBS spectra from different bacteria.



DFA is a statistical analysis used to predict a categorical dependent variable by one or more continuous or binary independent variables. It uses the spectral data with their group assignments and discriminates them by maximizing between-class variance using a unique set of constructed discriminant functions.



PLSDA is a multivariate inverse least squares discrimination method used to classify samples. It is used to find the maximum variance or separation between classes and not the variance of the data set. PLSDA is designed to work best with a "yes or no" test.

Experiment: 40 for each tested of each. 4 classes tested in 2 chemometric algorithms
Truth Table

Conclusion:
DFA techniques work well when all samples are known and exceeds PLSDA if groups to be discriminated are very similar.
PLSDA techniques work well when new samples are introduced into the test set and when a yes or no result is desired.

Conclusions and Future Work

The protocols developed in this experiment (cleaning of cone, cleaning of plate) will be integrated as a part of the current testing protocol, which will **reduce the limit of detection** of bacteria with **LIBS**.

Dual centrifugation and the use of two filters of different pore sizes will be tested on **yeast cells** to simulate a fluid suspension with contaminant through the metal cone.

The addition of a '**drying stage**' will be investigated.

The limits of detection will be determined for bacteria **swabbed off of surfaces** as well as **deposited in fluid suspensions** (both simulating clinical tests).

The **discrimination of different bacteria** when swabbed off of surfaces and deposited in fluid suspensions in the cone will continue to be investigated.

Testing of actual clinical specimens obtained from pathology labs will be initiated including bacteria suspended in complex mixtures (i.e. blood, saliva, cerebral spinal fluid). Other areas of interest include automation techniques to make the technique more usable by non-experts and more safe, without loss of sensitivity or specificity.

Acknowledgements

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References

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[3] - D.J. Malenfant, *Influences on the Emissions of Bacterial Plasmas Generated through Nanosecond Laser-Induced Breakdown Spectroscopy*, University of Windsor, Windsor, 2016.