Developments in the Rapid Diagnosis of Bacterial Pathogens Using Laser-Induced Breakdown Spectroscopy

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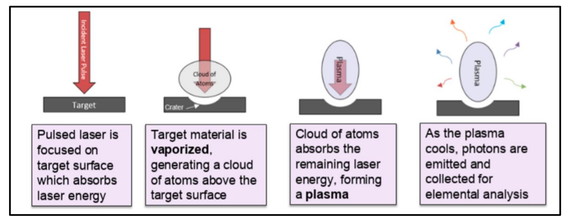
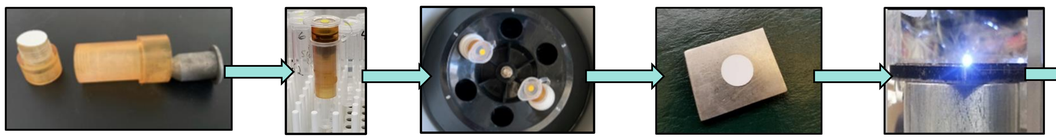
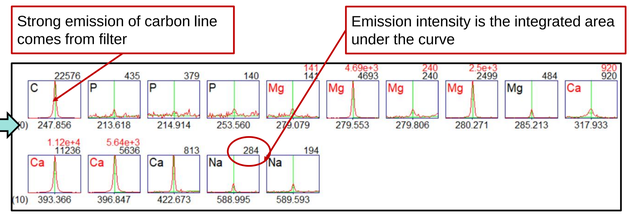
Introduction

Laser-induced breakdown spectroscopy (LIBS) is a rapid spectrochemical technique in which a pulsed laser ablates a substance to produce a microplasma. Light is collected from the microplasma by an optical fibre and dispersed by an Echelle spectrometer. The resulting spectrum provides an assay of the elemental composition of the substance. Our lab is developing a way to diagnose bacterial infections using LIBS. Previous work in this field has demonstrated that single cells can be identified when they are introduced via gas stream (2003). In 2007, Merdes et al. successfully applied principle component analysis (PCA) to discriminate between bacterial spores and contaminants such as pollen. In 2011 Gottfried et al. successfully applied chemometrics and down-selection of variables to discriminate between 5 species of bacteria. At the same time, Rehse et al. was using variable down-selection and discriminant function analysis (DFA) to discriminate between 5 species and 13 strains of bacteria. More recently, Rehse et al. showed that altering the membrane chemistry of bacterial cells plays a role in LIBS[1]. Now, our lab is working on reducing the limit of detection (LOD) and achieving reliable discrimination with cell counts similar to infectious cell counts. In addition, we are attempting to reliably detect and diagnose bacteria disease in sterile clinical fluids, such as blood and urine.



Methods

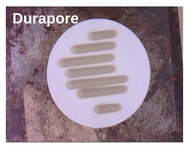




Phosphorus lines are highly indicative of bacteria. Na, Ca, and Mg also indicative of bacteria. All of these elements exist in the bacterial cell membrane.

Filter Media Study - Reducing Spectral Background

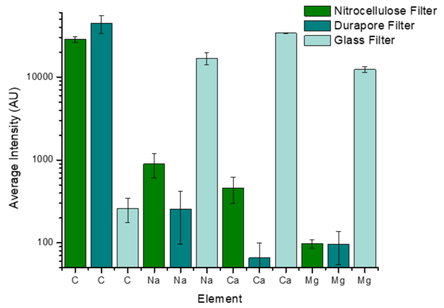
- Uniform Surface
- Ca, Mg and Na present
- Significant C emission
- Current medium for our LIBS testing procedure
- Nitrocellulose membrane



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

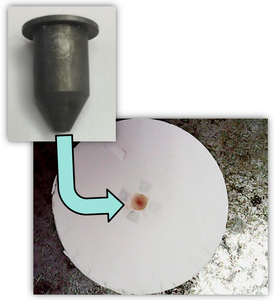
- Significant increase in Ca, Mg, and Na
- Significant reduction in C emission
- Destruction of testing surface after laser ablation

The filters used by our group have a significant carbon line emission. To reduce this carbon emission, other types of filters were tested using the LIBS apparatus.



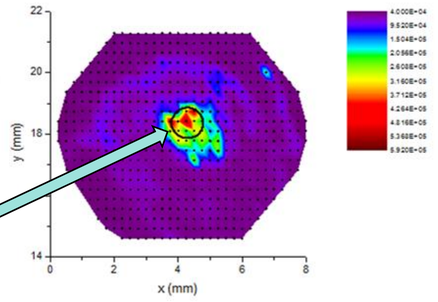
Conclusion: Nitrocellulose filters are the media with the lowest background signal. Durapore filters had lower carbon emission but higher emissions for all other measured ion lines. Glass filters had lower emission in carbon but higher emission in all lines important to bacterial identification. Na, Mg, Ca are all important to bacterial identification, therefore nitrocellulose filters were the best choice for bacterial deposition.

Reducing LOD - Concentrating Bacterial Cells



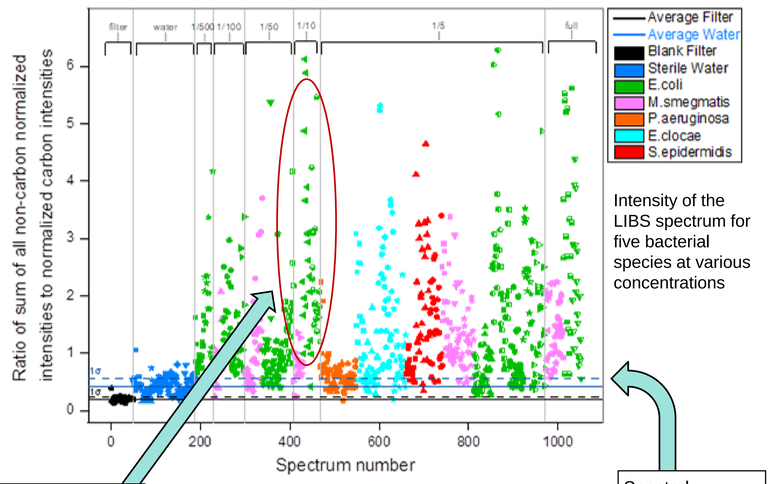
Bacteria deposited on center of filter concentrated by cone.

A cone was designed to concentrate bacteria on filter media during centrifugation to lower the limit of detection (LOD). The single-shot LOD without metal cone concentration was ~50000 CFU/mL. Using the metal cone, the single-shot LOD was calculated to be 5530 ± 872 CFU/mL.



Intensity map of deposition site of bacteria on filter. The red indicates higher intensity LIBS signal.

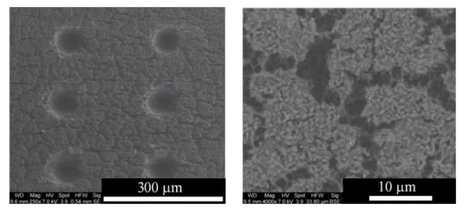
LIBS Spectral Intensity [2]



Intensity of the LIBS spectrum for five bacterial species at various concentrations

Shot-to-shot scatter attributed to non-uniformity of deposited bacterial films (shown in SEM)

SEM Images of Laser Ablated *S. epidermidis*

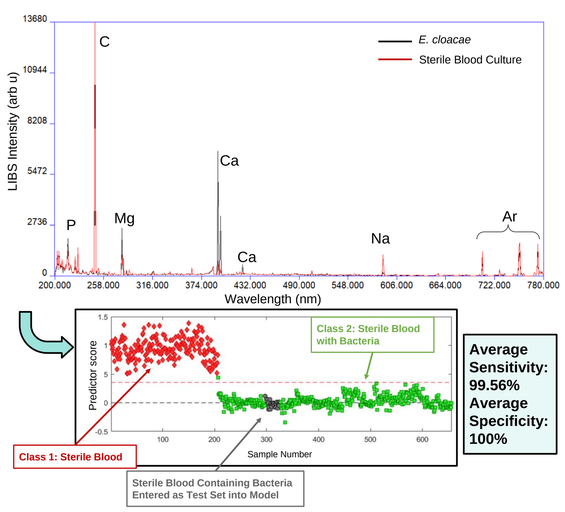


Spectral intensities below this threshold, consistent with water, eliminated from analysis. All intensities above are considered to be consistent with bacteria.

Ongoing research question:
How do we reduce shot-to-shot scatter and increase spectral intensity?

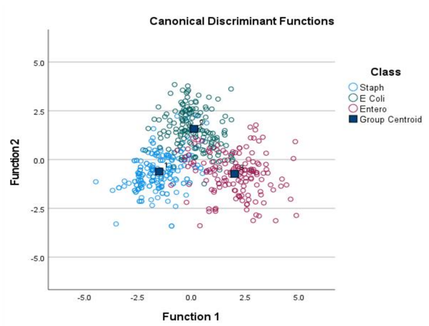
Detection and Diagnosis of Bacteria in Blood

- Sterile blood was prepared using the method above and characterized using our LIBS apparatus.
- Sepsis was simulated by 'spiking' sterile blood with pre-prepared bacterial suspensions which was deposited on filter media. Spectra were collected with the LIBS apparatus.
- External validation tests were performed with partial least squares discriminant analysis (PLSDA) to determine if we could detect the presence of bacteria in blood.
- Tests were also performed with DFA and an artificial neural network (ANN) to determine if we could discriminate between species of bacteria when detected in blood.



Average Sensitivity: 99.56%
Average Specificity: 100%

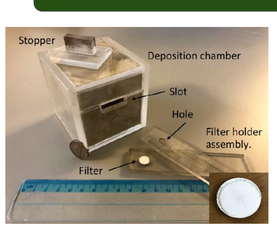
DFA Results

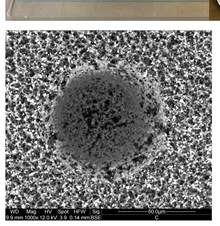


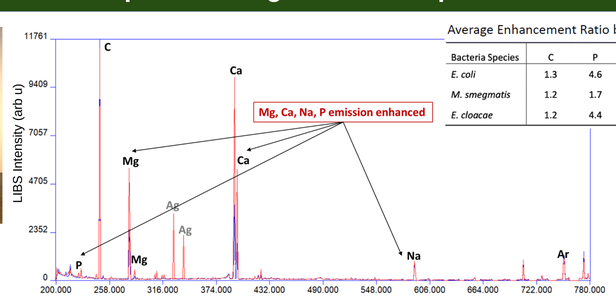
ANN Results

Sample Type	Sensitivity	Specificity
Sterile Blood Containing S. aureus	90.0%	85.0%
Sterile Blood Containing E. coli	76.7%	93.3%
Sterile Blood Containing E. cloacae	83.3%	96.7%

Enhancement of Spectra Using Silver Microparticles [3]





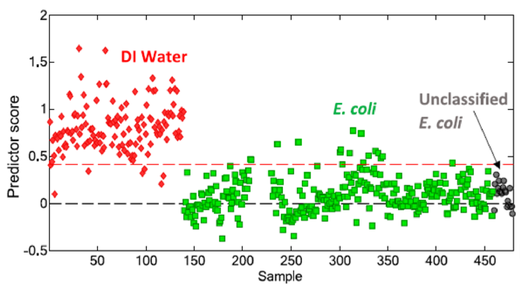


Average Enhancement Ratio by Element and Species

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

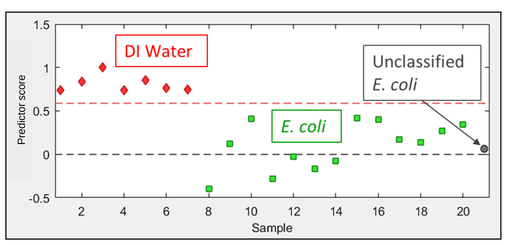
It has been shown that Ag and Au nanoparticles enhance LIBS emission, resulting in more intense spectra. Microparticles were investigated by our group to determine if they also cause enhancement, because they are cheaper, easier to obtain, and have never been investigated before. A chamber was built to uniformly deposit silver microparticles (top left). Uniform deposition was achieved as shown in the scanning electron micrograph (bottom left), and enhancement was observed for all lines used in bacteria identification, shown in the spectrum and table above.

PLSDA - Detecting Bacteria [2]



Individual Spectra
External validation was performed by withholding one filter at a time and testing that filter against a PLSDA model constructed from single-shot spectra. *E. coli* filters were withheld to test sensitivity and water filters were withheld to test specificity.

Add 30 samples to give one measurement per filter



Add-all Spectra
A summed filter was created by summing the intensity of each line after measurement from raw data. A PLSDA model was constructed and external validation was performed.

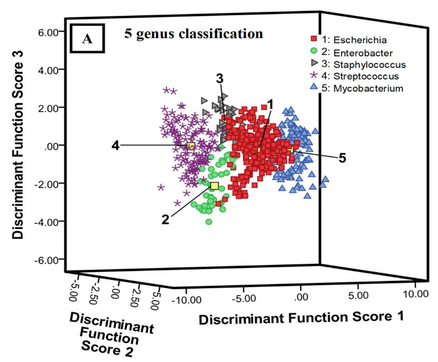
	Single-Shot PLSDA Model	Single-Shot External Validation	"Summed" Filters
Sensitivity	95%	87%	100%
Specificity	93%	93%	100%

✓ We can discriminate between spectra from a single shot of bacteria and sterile water with good accuracy

✓ After summing single shots, we can discriminate between bacteria and sterile water reliably

Sensitivity = (True Positives)/(True Positives + False Negatives)
Specificity = (True Negatives)/(True Negatives + False Positives)

DFA - Diagnosing Bacteria



Sensitivity: 91.37 ± 16.39 %
Specificity: 97.46 ± 9.35 %

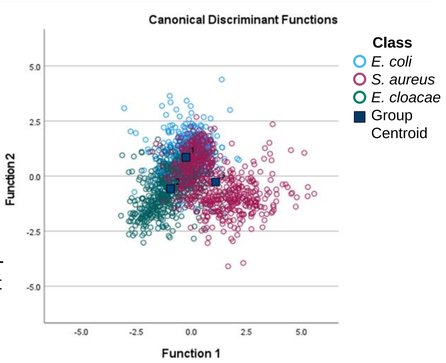
✓ We can reliably discriminate between species with high sensitivity and specificity when ablating high numbers of cells

✓ Result has been confirmed by other groups[4]

Question:
How does discrimination perform when there are fewer cells?

This DFA performs worse than the previous DFA. This could be due to several possible factors:

- There are fewer bacterial cells (simulating clinical infection)
- The background is different: the previous test had only a nutrient-free agar substrate, not a nitrocellulose filter.



	E. coli	S. aureus	E. cloacae
Sensitivity	73.28 %	64.93 %	60.10 %
Specificity	71.78 %	84.93 %	92.53 %
Classification error	27.47 %	25.07 %	23.68 %

Conclusions & Future Work

Conclusions

✓ Reduced limit of detection using custom-fabricated cone
✓ Achieved reliable discrimination between bacteria and sterile water by averaging single-shot spectra
✓ Achieved reliable discrimination between bacteria species when ablating large numbers of cells
✓ Achieved spectral enhancement using silver microparticles
✓ Achieved reliable detection of bacteria in blood

Future Work

➔ Improve discrimination between species in sterile fluids, including urine and cerebral spinal fluid
➔ Investigate the use of an ANN and full-spectrum analysis
➔ Develop a two-step process for detection of bacteria in sterile bodily fluids followed by diagnosis of bacterial infection
➔ Continue to investigate LIBS spectral enhancement using silver nanoparticles

References

1. S. J. Rehse, "Biomedical Applications of LIBS," *Laser-induced breakdown spectroscopy: Theory and applications*, vol. 182, Springer, 2016, pp. 457–485.
2. E. J. Blanchette, et al. "Detection and classification of bacterial cells after centrifugation and filtration of liquid specimens using laser-induced breakdown spectroscopy," *Applied Spectroscopy*, May 2022.
3. J. Marvin, E. J. Blanchette, S. Sleiman, H. Arain, and E. Tracey, "Silver Microparticle Enhanced Laser-Induced Breakdown Spectroscopy", *Applied Spectroscopy*, May 2022.
4. R. A. Putnam, Q. I. Mohaidat, A. Daabous, and S. J. Rehse, "A comparison of multivariate analysis techniques and variable selection strategies in a laser-induced breakdown spectroscopy bacterial classification," *Spectrochimica Acta Part B: Atomic Spectroscopy*, vol. 87, 161-167, 2013.