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

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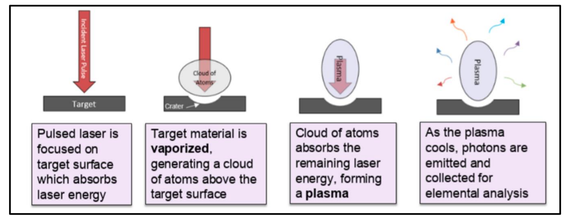
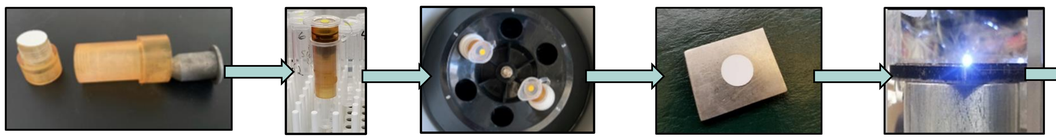
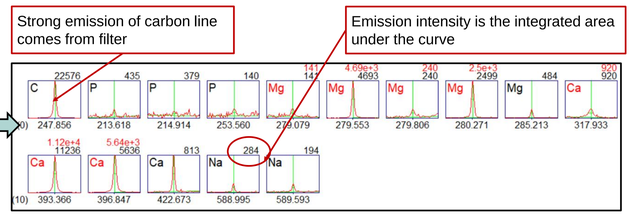
Introduction

Laser-induced breakdown spectroscopy (LIBS) is a rapid spectrochemical technique where a laser interacts with 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 chemical 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. Work on discriminating bacteria species from each other began with Gottfried et al. when they successfully used PLSDA 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 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 huge 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.

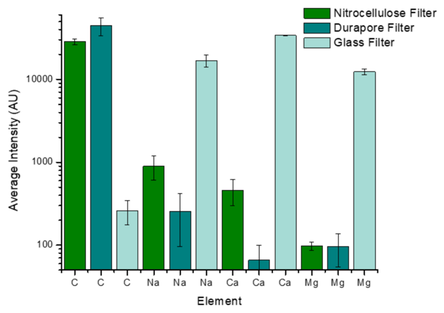
Filter Media Study - Reducing Spectral Background

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.

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

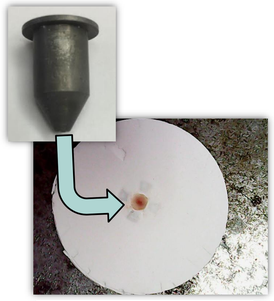
- Uniform Surface
- Reduced Ca, Mg, and Na
- **C emission increased**
- Scorching after laser ablation
- (PVDF) Hydrophilic Polyvinylidene Fluoride

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



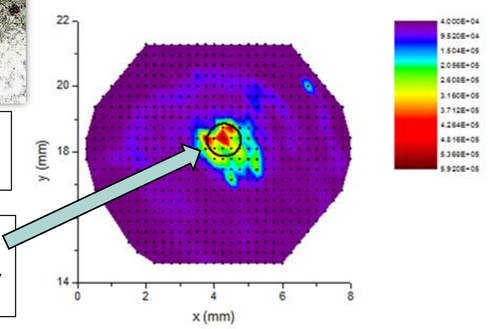
Conclusion: Nitrocellulose filters are the media with the lowest background signal. Durapore filters had lower carbon emission but higher emissions for all other measured ion lines. Glass filters had lower emission in carbon but higher emission in all lines important to bacterial identification. Na,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

Reducing LOD - Concentrating Bacterial Cells



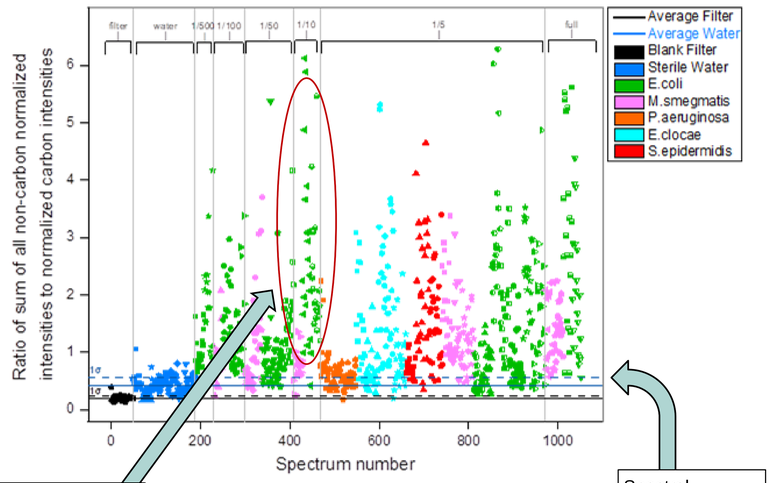
Bacteria deposited on center of filter concentrated by cone.

A cone was designed to deposit and bacteria on filter media to lower the limit of detection (LOD). The LOD without deposition using the metal cone was ~50000 CFU/mL per laser ablation event, after using the metal cone the LOD was calculated to be 5530 ± 872 CFU/mL per laser ablation event.



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

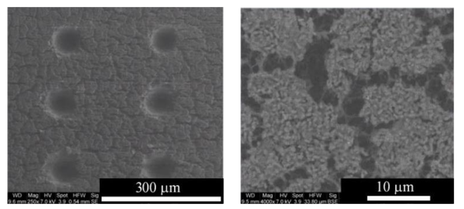
Current Total Library



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

Spectral intensities below this threshold, consistent with water, eliminated from analysis.

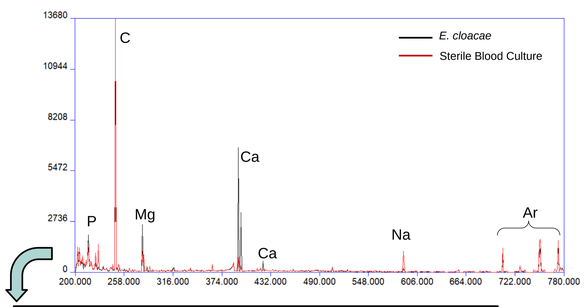
SEM Images of Laser Ablated *S. epidermidis*

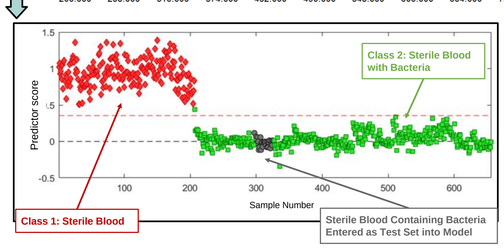


Ongoing research question:
How do we reduce shot-to-shot scatter and increase spectral intensity?

Detection and Diagnosis of Bacteria in Sterile Clinical Fluids

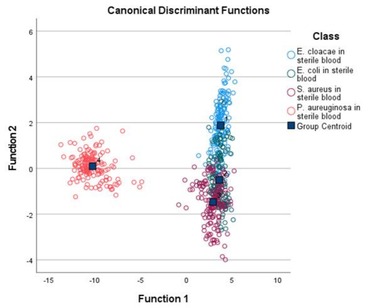
Sterile clinical specimens (blood) were prepared using the method above and characterized using our LIBS apparatus. Sepsis and UTI's simulated by 'spiking' sterile clinical specimens (blood) with pre-prepared bacterial suspensions. This solution was deposited using the method above.





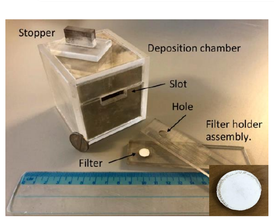
Average Sensitivity: 99.56%
Average Specificity: 100%

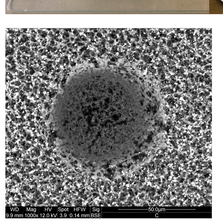
DFA Results vs. ANN Results

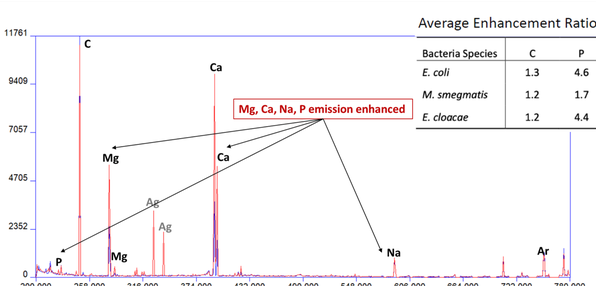


Sample Type (ANN)	Sensitivity	Specificity
Sterile Blood Containing *P. aeruginosa*	97.2 %	100 %
Sterile Blood Containing *S. aureus*	100 %	100 %
Sterile Blood Containing *E. coli*	100 %	100 %
Sterile Blood Containing *E. cloacae*	100 %	98.9 %
Sample Type (DFA)	**Sensitivity**	**Specificity**
Sterile Blood Containing *P. aeruginosa*	98.7 %	100 %
Sterile Blood Containing *S. aureus*	84.0 %	92.9 %
Sterile Blood Containing *E. coli*	74.7 %	93.6 %
Sterile Blood Containing *E. cloacae*	78.0 %	92.0 %

Enhancement of Spectra Using Silver Microparticles





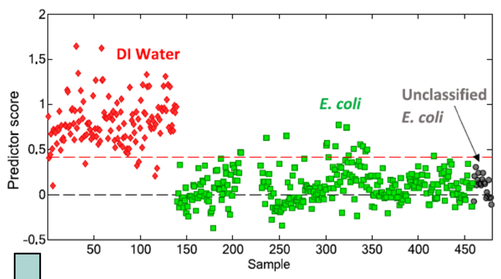


Average Enhancement Ratio by Element and Species

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 bigger spectra. Microparticles were investigated by our group to determine if they also cause enhancement. A chamber was built to uniformly deposit silver microparticles (top left). Uniform deposition was achieved as shown by SEM image, and enhancement was observed for all lines used in bacteria identification, shown in the spectrum and table above.[3]

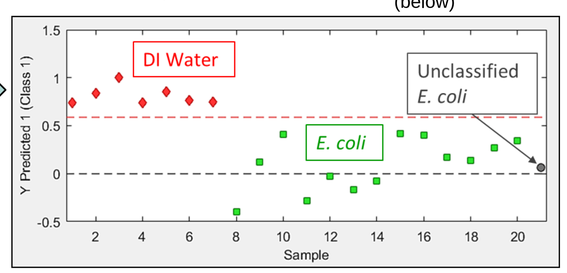
PLSDA - Detecting Bacteria



Individual Spectra
External validation of single spectra (left)

Summed Spectra
External validation of summed spectra (below)

Add 30 samples to give one measurement per filter



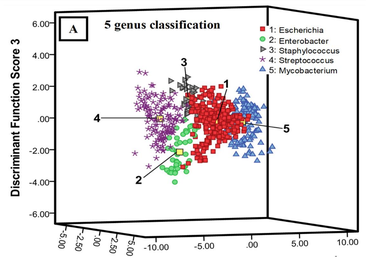
	Single-Shot PLSDA Model	SIngle-Shot External Validation	"Summed" Filters
Sensitivity	95%	87%	100%
Specificity	93%	93%	10%

✓ **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[4]**

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

DFA & ANN- Diagnosing Bacteria

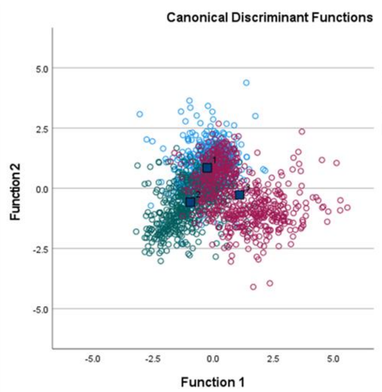


Sensitivity: 91.37 ± 16.39 %
Specificity: 97.46 ± 9.35 %

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

✓ Result has been confirmed by other groups[2]

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



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

- There is less bacteria: previously, solid pellets were used
- The background is different: the previous test had no nitrocellulose filter (and no background)

DFA Results			
Sample	**Sensitivity (%)**	**Specificity (%)**	**Classification Error**
E. coli	73.28 %	71.78 %	27.46 %
S. aureus	64.93 %	84.93 %	25.06 %
E. cloacae	60.10 %	92.53 %	23.68 %
ANN Results			
Sample	**Sensitivity (%)**	**Specificity (%)**	**Classification Error**
E. coli	98.0 %	97.7 %	2.13 %
S. aureus	93.3 %	97.2 %	4.28 %
E. cloacae	91.2 %	96.1 %	6.33 %

Conclusions & Future Work

Conclusions

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

Future Work

➔ Improve discrimination between species in sterile fluids by utilizing summation method and applying ANN
➔ 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 with silver nanoparticles

References

1. S. J. Rehse, "Biomedical Applications of LIBS," in *Laser-induced breakdown spectroscopy: Theory and applications*, vol. 182, SPRINGER, 2016, pp. 457–485.
2. Russell A. Putnam, Qassem I. Mohaidat, Andrew Daabous, Steven J. Rehse (2013). A comparison of multivariate analysis techniques and variable selection strategies in a laser-induced breakdown spectroscopy bacterial classification. *Spectrochimica Acta Part B: Atomic Spectroscopy, 87*, 161-167. https://doi.org/10.1016/j.sab.2013.05.014
3. J. Marvin, E. Blanchette, S. Sleiman, H. Arain, E. Tracey, "Silver Microparticle Enhanced Laser-Induced Breakdown Spectroscopy", *Applied Spectroscopy*, pp. , (In-print May 2022).
4. 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*, pp. 1–11, May 2022.