Quantification of Sensitivity and Specificity in a Laser-Induced Breakdown Spectroscopy Diagnostic Assay for Pathogenic Bacteria Detection and Classification

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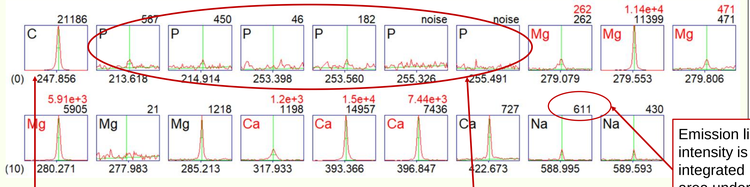
Introduction

We investigate the use of Laser-Induced Breakdown Spectroscopy (LIBS) to rapidly detect and diagnose bacterial pathogens. Clinical conditions are simulated by **swabbing bacteria off of a metal plate.** Bacteria are then centrifuged through a **metal cone** onto a nitrocellulose filter. Samples are ablated in an **argon environment**.

Ablation of the target with nanosecond laser pulses results in a **broadband emission spectrum** allowing us to determine the trace elements specific to each bacterial species. 19 emission lines from a LIBS spectrum of *E. coli* are shown below.

19 Lines From Optical LIBS Spectrum



Emission line intensity is integrated area under the curve

Strong emission from carbon line present in both bacteria and nitrocellulose filter.

Work is ongoing to increase the intensity of the phosphorus lines which are strong indicators of bacteria.

Motivation

Our goal is to be able to accurately detect and classify the smallest number of cells possible. This research focuses on:

- investigating the efficacy of chemometric algorithms;
- minimizing classification error using DFA;
- summing spectra from each filter.

Results: Chemometric Analysis - Algorithm Comparison

Chemometric analysis is a technique used to classify unknown spectra by constructing models from known data. 164 independent variables were created from the 19 LIBS emission lines in each spectrum, which were used in various chemometric algorithms.

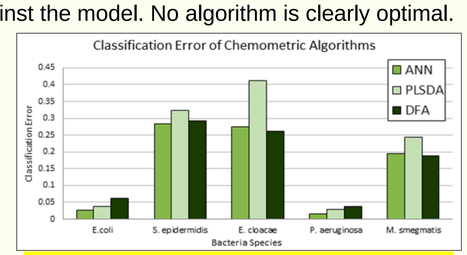
Pseudodata

A large Gaussian pseudodataset was created to test different algorithms. For each of the 19 emission lines, the mean of the single shot spectra was calculated. Then, a Gaussian noise filter was applied to create 998 spectra normally distributed about the mean with a standard deviation of 250.

Algorithm Comparison

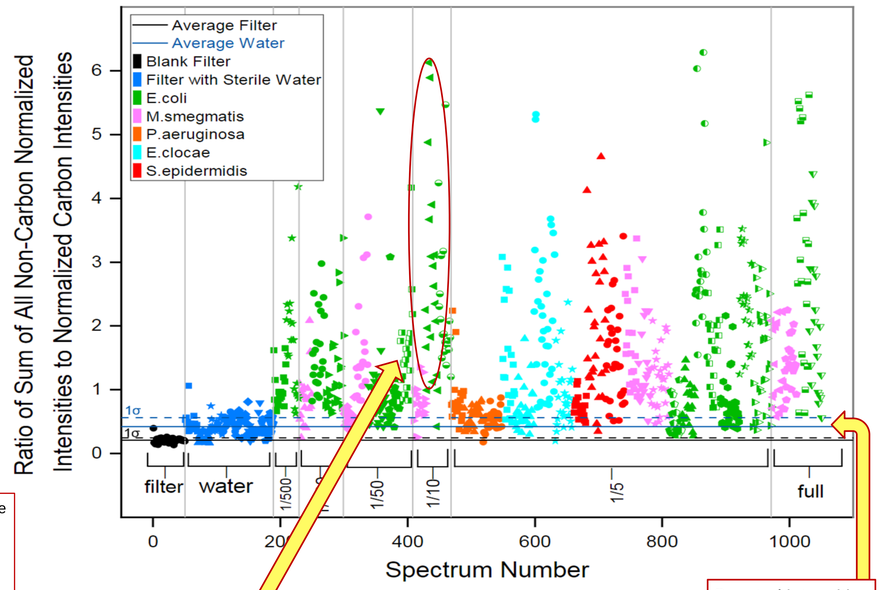
ANN, DFA and PLSDA algorithms were compared. 800 pseudodata points were selected at random to create the training model and the remaining 199 points were tested against the model. No algorithm is clearly optimal.

DFA was chosen to discriminate between all 5 classes of bacteria because it can be done much faster than ANN and is superior to PLSDA. Work is ongoing to test the use of ANN.



The classification error combines the sensitivity and specificity
Classification error = 1-(sensitivity+specificity)/2

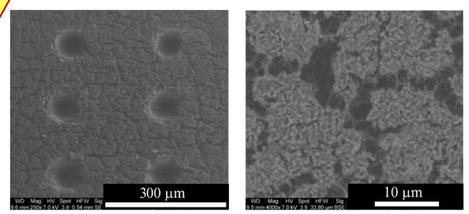
LIBS Spectral Intensity as a Function of Bacterial Concentration (5 Species of Bacteria)



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*



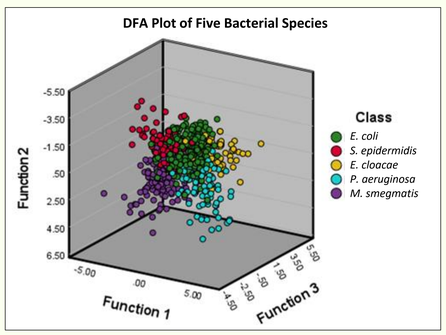
Results: DFA - Classification of Bacteria By Species

862 individual spectra from all 5 species were entered into a discriminant function analysis (DFA) model which reduced the 164 independent variables to 4 discriminant function scores to maximize the variance between bacteria classes.

10 Fold Cross Validation:

The spectra were randomly separated into 10 groups. 9 groups were used to train the discrimination model and the tenth used for validation. This was repeated 10 times so each group was used for validation.

Bacteria	Sensitivity	Specificity	Classification Error
E. coli	60%	79%	31%
S. epidermidis	64%	91%	23%
E. cloacae	50%	91%	29%
P. aeruginosa	66%	94%	20%
M. smegmatis	65%	82%	27%



Work is ongoing to improve classification of all 5 species using the addall spectra (explained at right) to eliminate scatter between individual shots.

Results: PLSDA - Detection of Bacteria

Partial Least Squares Discriminant Analysis (PLSDA):

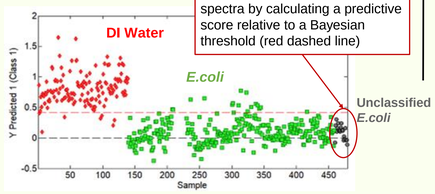
- 14 filters of *E. coli* (various concentrations); 7 filters of water
- 20-30 single-shot spectra/filter
- 320 total *E. coli* spectra, 139 total water spectra

Individual Spectra

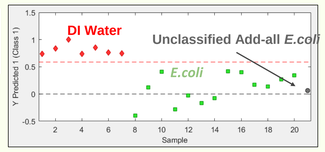
A PLSDA model was constructed out of all single-shot LIBS spectra. External validation was performed by withholding one filter at a time and testing that filter against the model. *E. coli* filters were withheld to test the sensitivity and water filters were withheld to test the specificity.

	PLSDA Model	External Validation
Sensitivity	95%	87%
Specificity	93%	93%

PLSDA categorizes unknown spectra by calculating a predictive score relative to a Bayesian threshold (red dashed line)



"Add-All" External Validation



Add-all Spectra

All spectra on each filter were averaged to produce 1 spectrum per filter. Two methods were used to calculate the average:

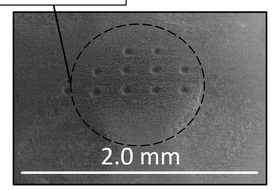
1. Intensity of each line averaged after measurement from raw data
2. All spectral raw data averaged prior to line intensity measurement

A PLSDA model was constructed using the 21 averaged filter spectra and external validation was performed for both methods.

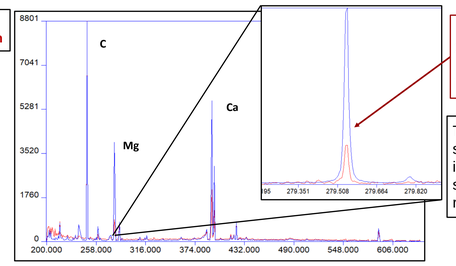
	"Add-all" Filters	"Summed" Filters
Sensitivity	93%	100%
Specificity	86%	100%

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

Circular area of filter containing 10 single-shot spectra.



"Add-all" Spectrum
Single-Shot Spectrum



Magnesium 279 nm line has greater intensity

The Add-all spectra increases the signal to noise ratio

Conclusions and Future Work

- Shot-to-shot variation requires summing all spectra obtained from a filter prior to discrimination. We will continue to use this method for detection of bacteria
- ANN and DFA are better algorithms for discrimination between bacterial species, and the use of ANN will be investigated further

Future work:

- Investigating multiple data analysis techniques prior to chemometric analysis (e.g. use of raw data instead of normalized data, subtracting an average blank filter spectrum prior to analysis)
- Use of metal microparticles deposited on filter to enhance LIBS emission
- Effect of ultra-pure water on the background signal
- Explore ways to make bacterial deposition more uniform

Acknowledgements

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References

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[2] S.J. Rehse, A Review of the Use of Laser-Induced Breakdown Spectroscopy for Bacterial Classification, Quantification, and Identification, Spectrochim. Acta B **154** (2019) 50-69.
[3] D.J. Malenfant, *Influences on the Emissions of Bacterial Plasmas Generated Through Nanosecond Laser-Induced Breakdown Spectroscopy*, University of Windsor, Windsor, 2016.