Toward the development of a rapid diagnostic test for bacterial meningitis using laser-induced breakdown spectroscopy

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Motivation

Bacterial meningitis, an infection of the brain and spinal cord's protective layers, typically requires a spinal tap for diagnosis, with results taking up to 3 days [1]. Delays and inappropriate antibiotic use can lead to severe complications, including irreversible brain damage or death [2].

Laser-induced breakdown spectroscopy (LIBS) offers the potential for rapid and accurate pathogen identification with minimal sample preparation, improving diagnostic efficiency and patient outcomes.

Custom 3D-Printed Centrifuge Insert

Old Design

Orange pieces screwed together with filter in between; separate metal cone inserted for bacteria concentration.

x Metal cone piece not firmly placed in insert consistently, unable to create a tight seal

x Indent in filter only on four corners, unable to fully contain bacteria

Inconsistent and low bacterial signal

New Design

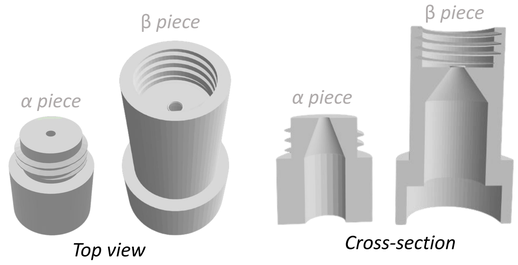
Two components, with a built-in concentration cone.

✓ Concentrates bacteria in a 1 mm diameter circle

✓ The ridge on the aperture of β piece prevents bacterial escape, allowing only water and ions to diffuse

✓ Indent created on filter is more easily identifiable by the LIBS apparatus and seals bacteria

Stronger bacterial signal and more reproducible data

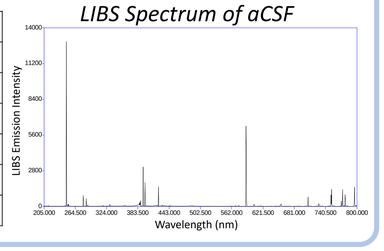


Artificial Cerebrospinal Fluid

Cerebrospinal fluid (CSF) is the fluid the brain, cranium, and spinal cord is bathed in. **Artificial cerebrospinal spinal fluid (aCSF)** closely matches the electrolyte concentrations of actual CSF and is prepared from analytical grade reagents and high purity water.

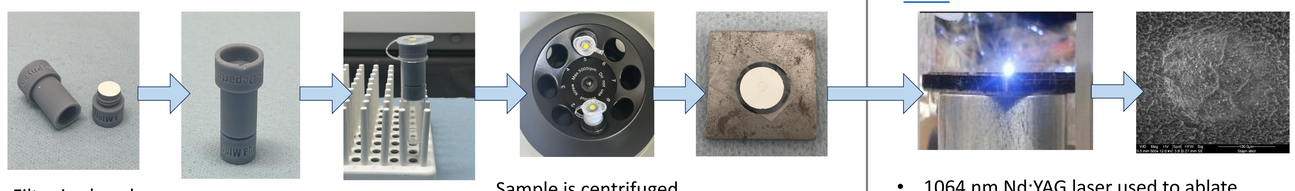
Ion	Concentrations (mM)
Na^+	150
K^+	3.0
Ca^{2+}	1.4
Mg^{2+}	0.8
P^{3-}	1.0
Cl^-	155

LIBS Spectrum of aCSF



Methodology

Sample Preparation



Filter is placed on centrifuge insert α.

β is screwed in, facing down, until tight. When assembled, it is placed into a test tube and 100 µL of bacterial dilution is pipetted in ten 10 µL aliquots through the large aperture in β.

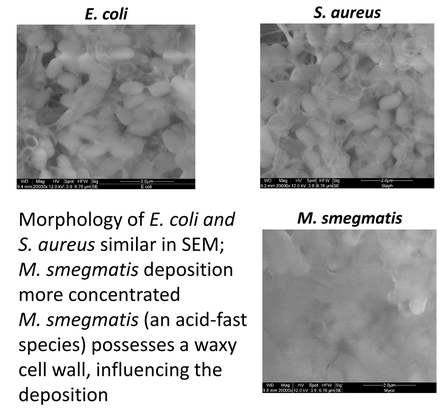
Sample is centrifuged at 2400 rpm for 5 min allowing for aCSF to drain through the filter while concentrating the bacteria.

Once dry, filter is mounted onto a steel piece with double-sided tape.

LIBS

- 1064 nm Nd:YAG laser used to ablate surface of target
- Laser energy absorbed to create plasma
- Photons emitted from plasma are collected and dispersed using an Echelle spectrometer
- Production of an optical emission spectrum, resulting in an elemental analysis of target

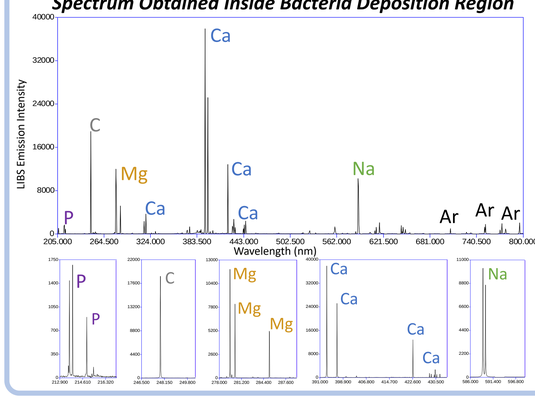
SEM Images



- Morphology of *E. coli and S. aureus* similar in SEM; *M. smegmatis* deposition more concentrated
- *M. smegmatis* (an acid-fast species) possesses a waxy cell wall, influencing the deposition

Spectral Analysis

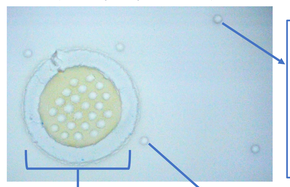
Spectrum Obtained Inside Bacteria Deposition Region



- **P** emissions lines are highly indicative of the presence of bacteria
- **Ar** lines from the chamber environment the samples are ablated in
- **C** line primarily comes from nitrocellulose filter
- **Mg** and **Na** also indicative of bacteria presence, although they are also present in aCSF in a lower concentration
- **Ca** lines are very strong when bacteria is added to aCSF

Ratios of line intensities allow for bacteria identification and classification.

nitrocellulose filter (0.45 µm pore size)

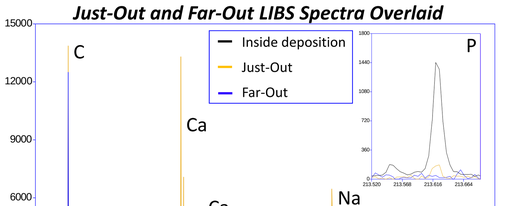


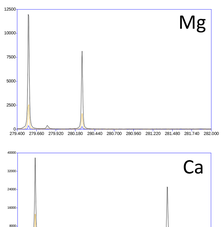
Laser shot obtained far from deposition region and near edge of filter or "Far-Out"

Bacterial deposition region bounded by custom cone indent

Laser shot obtained near to deposition region or "Just-Out"

Just-Out and Far-Out LIBS Spectra Overlaid





Cone indent constrains bacteria as no significant phosphorus emission is observed in outer regions, while aCSF diffuses out of the deposition region.

46 filters with 25 shots per filter

- 13 aCSF
- 11 *E. coli* + aCSF
- 11 *S. aureus* + aCSF
- 11 *M. smegmatis* + aCSF

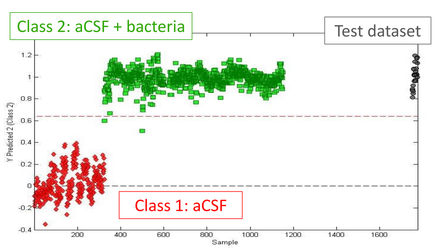
Results: PLS-DA

Partial least squares-discriminant analysis (PLS-DA) was used to calculate the likelihood of each sample belonging to a predefined class.

- **107 independent variables** inputted - 15 chosen emission lines and 92 ratios of those lines
- Each individual filter (consisting of 25 spectra) is withheld to create a **test dataset** that is compared against the remaining filters which make up the **model**

Two Class: Determine presence of bacteria

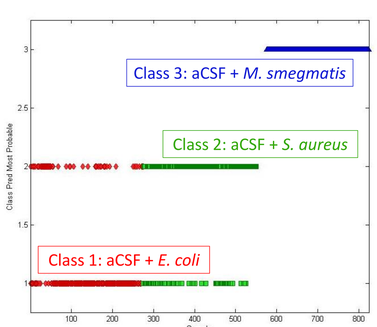
Sample	Average Sensitivity
Excluding aCSF	100%
Excluding aCSF + *E. coli*	99.27%
Excluding aCSF + *S. aureus*	100%
Excluding aCSF + *M. smegmatis*	100%



✓ *PLS-DA is capable of detecting bacterial infection.*

❑ *Is it now possible to identify the species of bacteria?*

Three Class: Diagnose bacterial identity



Sample	Average Sensitivity
Excluding aCSF + *E. coli*	58.91 %
Excluding aCSF + *S. aureus*	58.55 %
Excluding aCSF + *M. smegmatis*	100 %

Many *E. coli* and *S. aureus* spectra failed to classify properly.

An alternative technique is required.

Results: PCA-ANN

Use an artificial neural network (ANN) to identify the bacterial species

Rather than entering the full LIBS spectra (200 – 590 nm) that contain 42,000 independent variables into the ANN, **principal component analysis (PCA)** preprocesses the data. The result is 10 PC scores that capture 99.58 % of variance. PCA and ANN scripts were implemented in Python.

Internal cross-validation (80:20 split) randomly selects 20% of the dataset to be tested against remaining 80% of spectra.

80:20 Cross-Validation Test Results				
	E. coli	*S. aureus*	*M. smegmatis*	Average
Sensitivity	95.8 %	96.0 %	100.0 %	97.2 %
Specificity	98.2 %	98.0 %	99.7 %	98.6 %
Classification Accuracy	97.4 %	97.3 %	99.8 %	98.2 %

External validation tests individually compare one filter (25 spectra) against left over spectra.

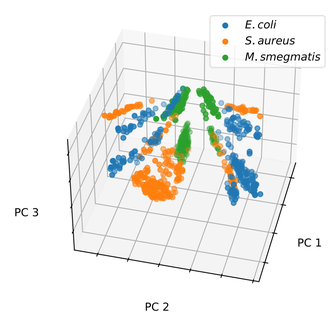
External Validation Test Results				
	E. coli	*S. aureus*	*M. smegmatis*	Average
Sensitivity	74.6 %	71.1 %	99.5 %	81.7 %
Specificity	87.6 %	87.9 %	97.0 %	90.9 %
Classification Accuracy	83.3 %	82.3 %	97.9 %	87.8 %

TP = True Positives FP = False Positives
TN = True Negatives FN = False Negatives

$$\text{Sensitivity} = \frac{TP}{TP + FN} \times 100\%$$

$$\text{Specificity} = \frac{TN}{TN + FP} \times 100\%$$

$$\text{Classification Accuracy} = \frac{TP + TN}{TP + TN + FP + FN} \times 100\%$$



The first three PC scores were plotted to visualize the variance in the data

Conclusions and Future Work

Conclusions

- Developed a custom centrifuge insert and new bacterial deposition method, increasing bacterial signal and reproducibility
- Achieved nearly 100% accuracy in distinguishing "sterile" vs. "spiked" aCSF using PLS-DA
- Implemented PCA-ANN models to identify specific bacteria in aCSF, with overall test specificity and sensitivity of 81.7% and 90.9 %, respectively

Future Work

- Improve PCA-ANN classification accuracy and investigate why *M. smegmatis* has better classification ability than *E. coli* or *S. aureus*
- Extend analysis to other bacterial strains, such as *M. tuberculosis* and *Streptococcus mitis*
- Continue to enhance data reproducibility by reducing variability due to sample preparation and mounting

[1] Slane, Valori H. and Chandrashekhar G. Unakal. "Tuberculous Meningitis." *StatPearls*, StatPearls Publishing, 18 November 2022.
[2] Telano, Lauren N. and Stephen Baker. "Physiology, Cerebral Spinal Fluid." *StatPearls*, StatPearls Publishing, 4 July 2023.