A laser-based method to diagnose bacterial meningitis

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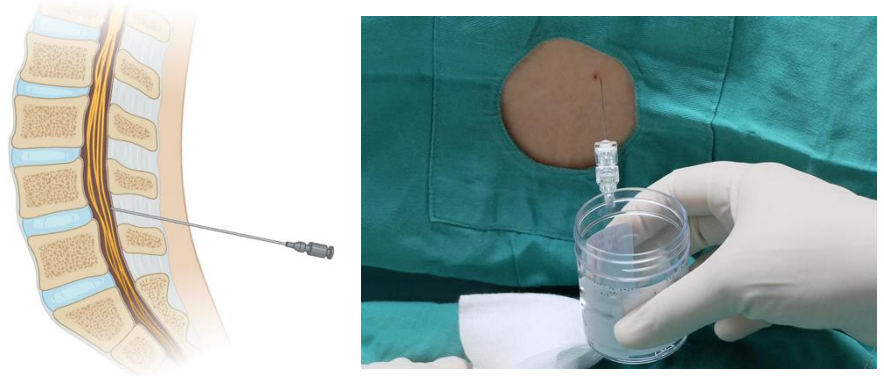
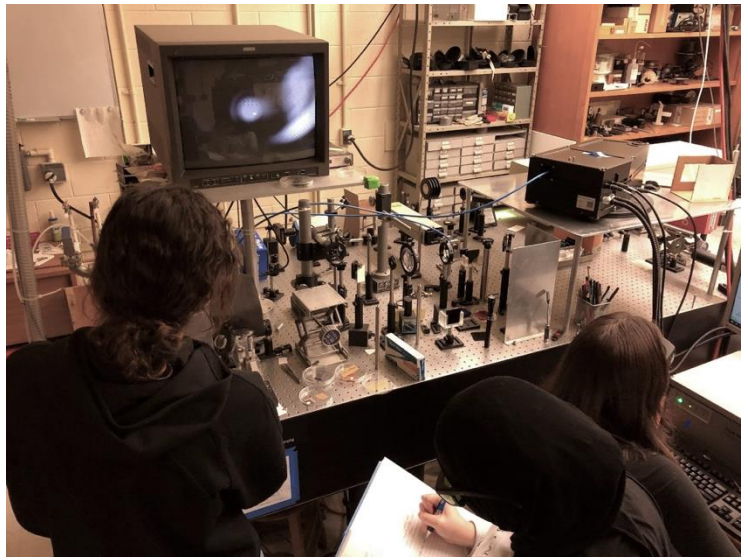
Introduction

- Bacterial meningitis
  - Infection of meninges
  - Delaying treatment ↑ risks by **30%** per hour[1]
  - Test cerebrospinal fluid (CSF) from lumbar puncture or “spinal tap”
- **L**aser-**I**nduced **B**reakdown **S**pectroscopy (**LIBS**)
  - ↓ diagnostic time, *days* → *minutes*
  - No culturing or genetic amplification



Lumbar Puncture / “Spinal Tap”



LIBS Laser Apparatus

[1] M. Glimåker, B. Johansson, M. Bell, M. Ericsson, J. Bläckberg, M. Brink, J. Sjölin, Early lumbar puncture in adult bacterial meningitis—rationale for revised guidelines. Scandinavian Journal of Infectious Diseases, (2013). 45(9), 657–663. https://doi.org/10.3109/00365548.2013.799289

Methods

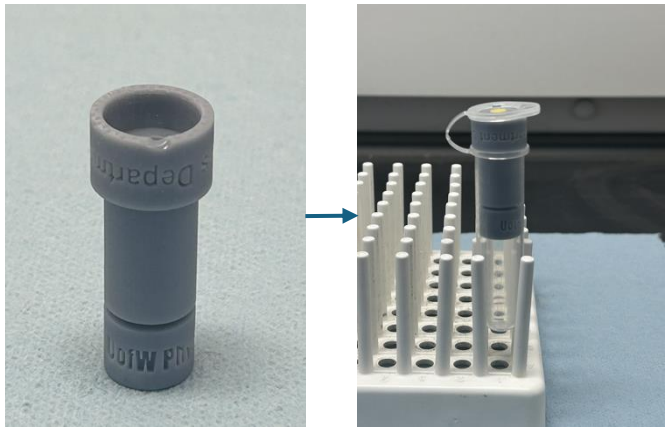
1. Preparation → **2. Deposition** → **3. Laser Ablation**

- artificial Cerebrospinal Fluid (aCSF)

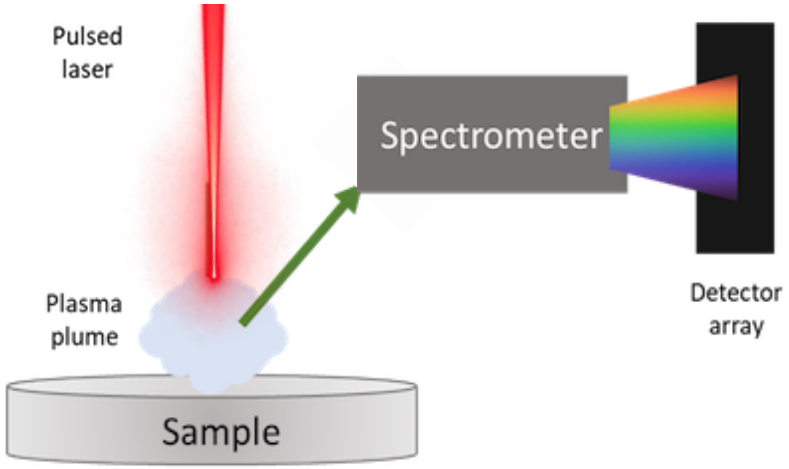
Bacteria

- *M. smegmatis, E. coli, S. aureus*

- Nitrocellulose filter, 0.45 µm pore size



- 3D-printed centrifuge tube insert
- centrifugation

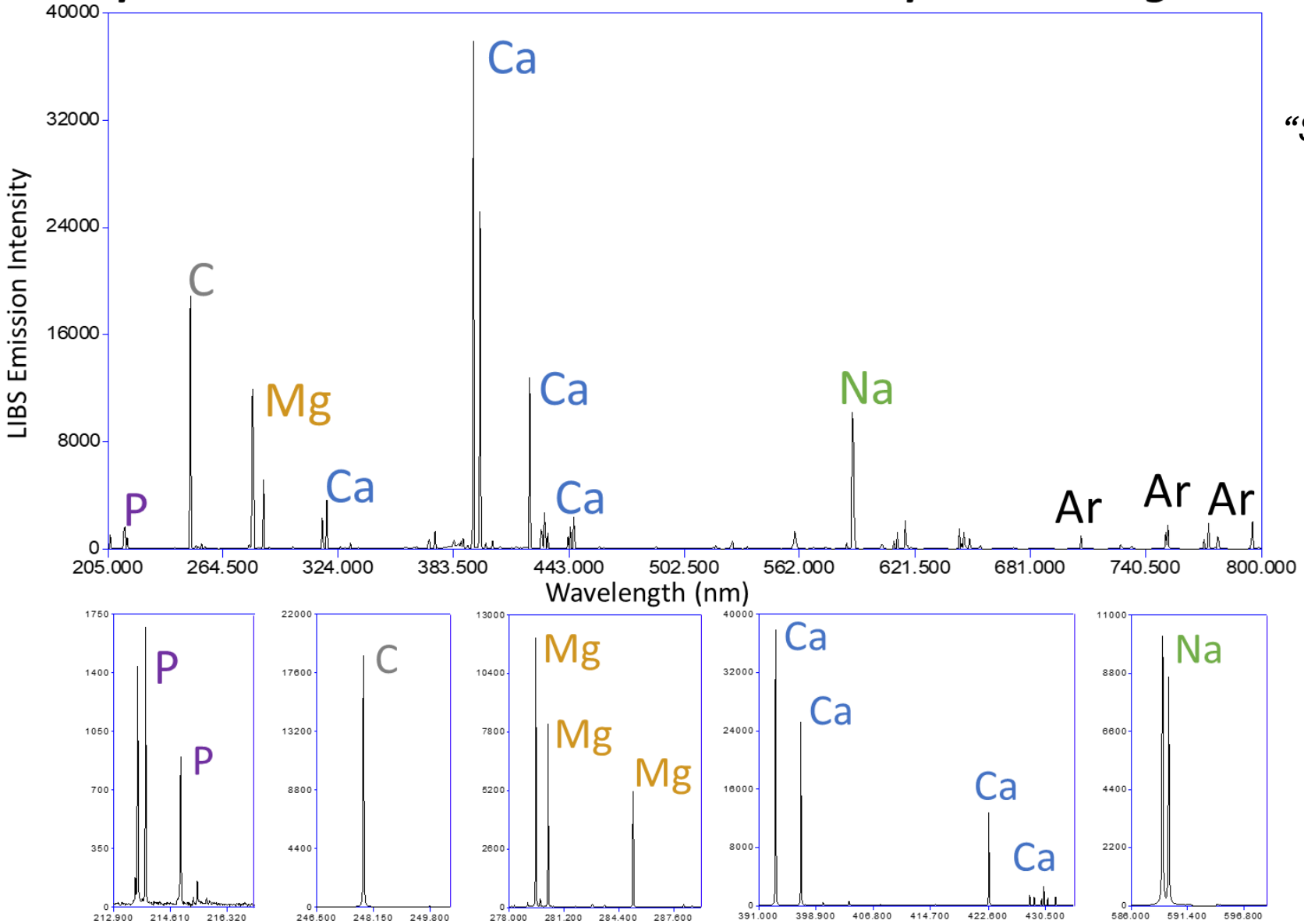


- Laser: 10 ns, 100 µm diameter
- can detect as low as 5000 cells per shot
- atomization and ionization

4. Analysis

- PLSDA
- PCA-ANN

Spectrum Obtained Inside Bacteria Deposition Region



"Spectral Fingerprint"

Analysis and Results

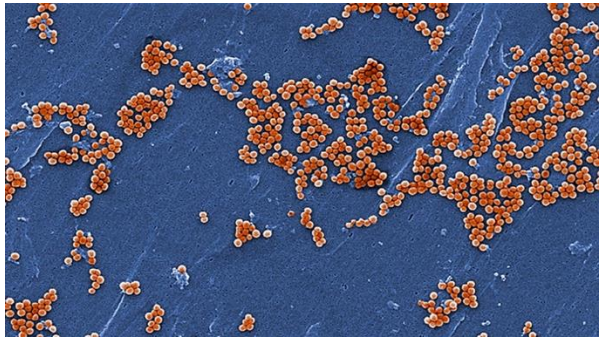
"Machine Learning"

	PLSDA (Partial-Least Squares Discriminant Analysis)		**PCA-ANN** (Artificial Neural Network with Principal Component Analysis pre-processing)
	Two-class	Three-class	
Test	To distinguish between spiked and not spiked aCSF	To distinguish between bacterial species	
Results	Sensitivity: ~100% Specificity: ~100%	Average Sensitivity: *M. smegmatis*: 100% *E. coli* and *S. aureus*: ~59%	*Average Sensitivity: 81.7% *Average Specificity: 90.9%
Conclusion	✅	❓	✅

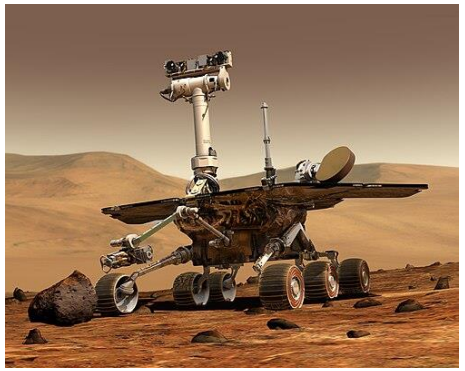
*averages across all 3 bacterial species: *Mycobacterium smegmatis, Escherichia coli, Staphylococcus aureus*

Conclusion

- Developed procedure
  - Rapid identification
  - Multiple-element analysis
  - Minimal sample prep
- Next steps
  - other bacterial species
  - lowest detectable concentration
- Impacts
  - LIBS: procedures & parameters
  - Healthcare: clinical device, ↑ diagnostic accuracy, ↓ wait times, ↓ antimicrobial resistance



Staphylococcus aureus

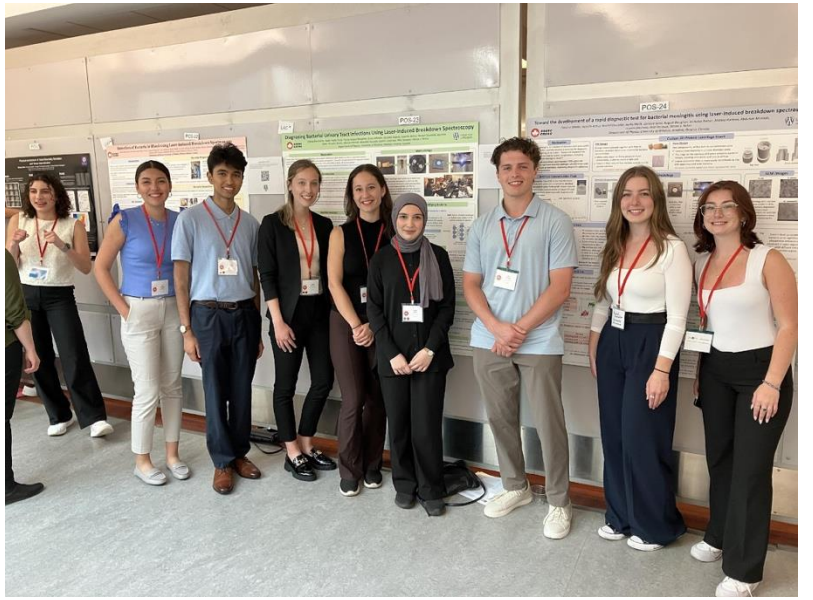


LIBS in hand-held devices and Curiosity Rover on Mars

Credits

- Dr. Steven J. Rehse
- The Rehse Group
- University of Windsor Outstanding Scholars program

www.uwindsor.ca/people/rehse/



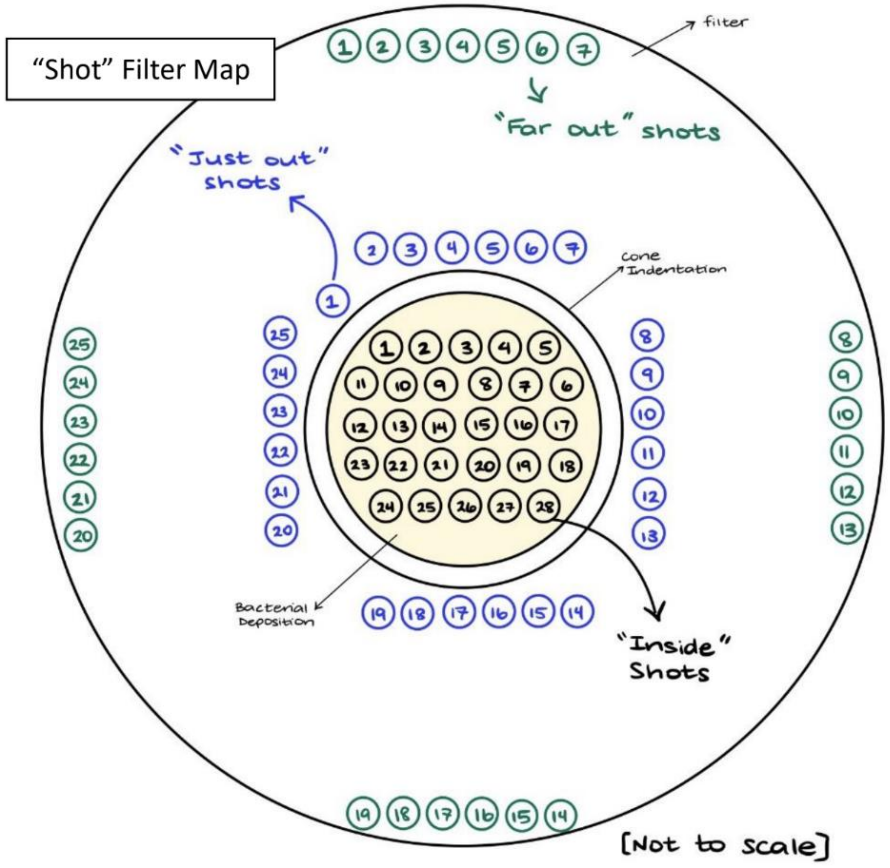
University of Windsor
Faculty of Science

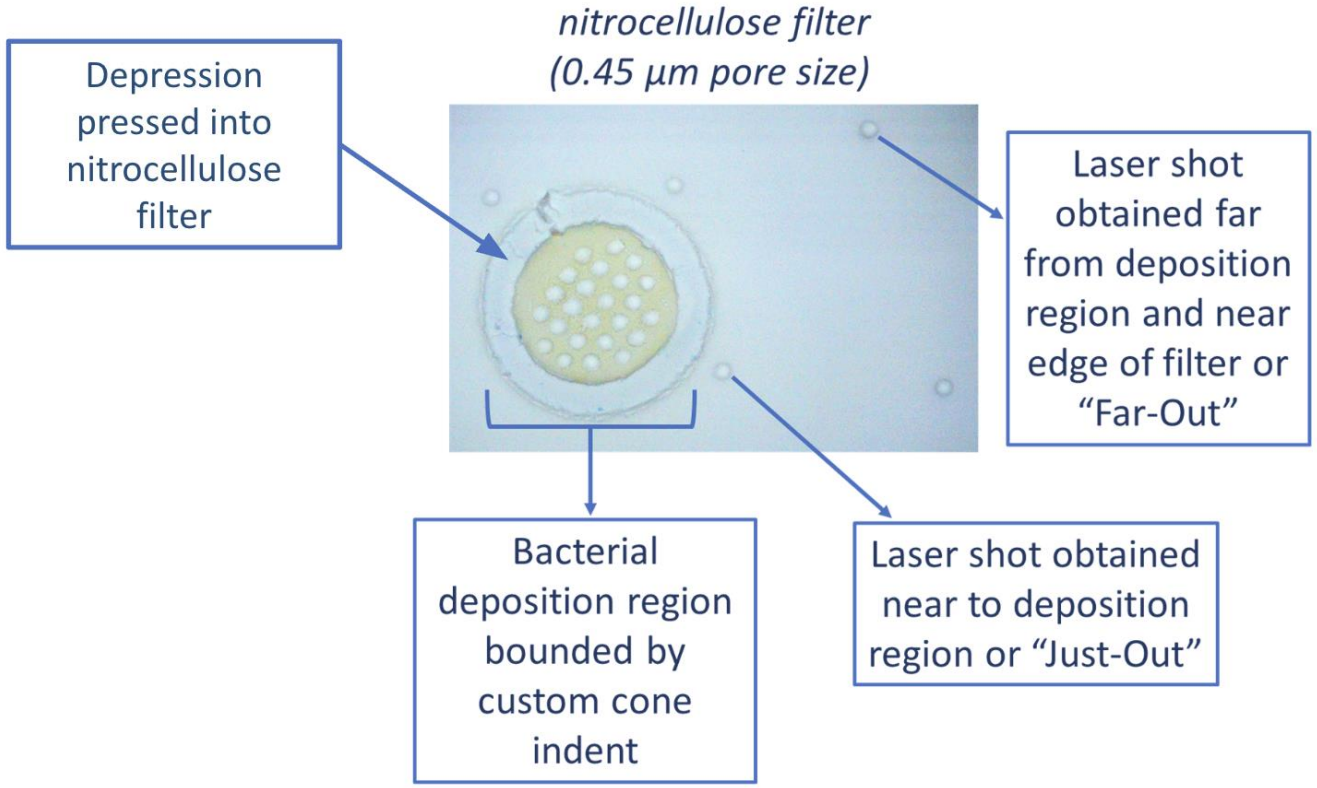
NSERC CRSNG

Ontario Research Fund

INNOVATION
Canada Foundation for Innovation
Fondation canadienne pour l'innovation

Extra Material

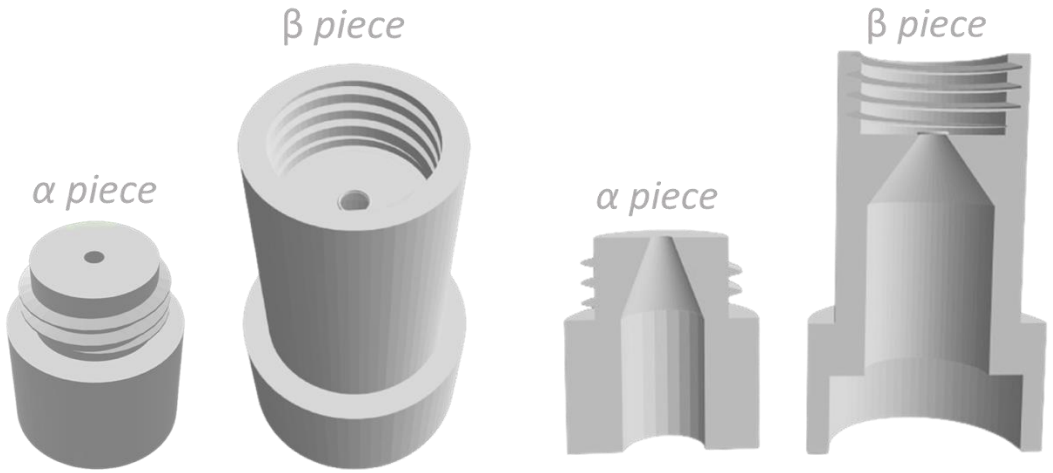




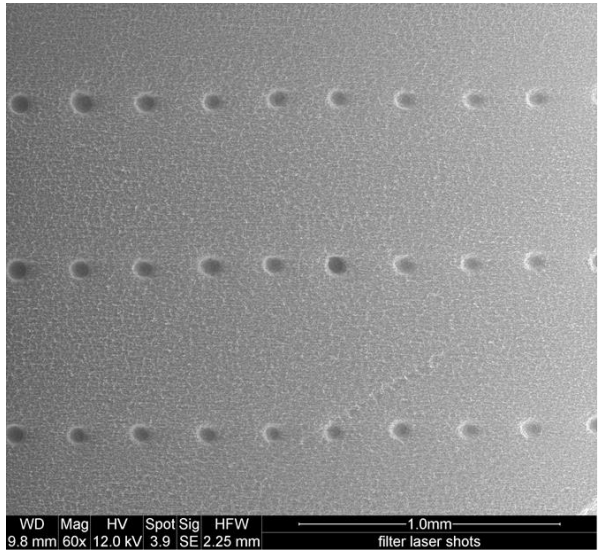
aCSF

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

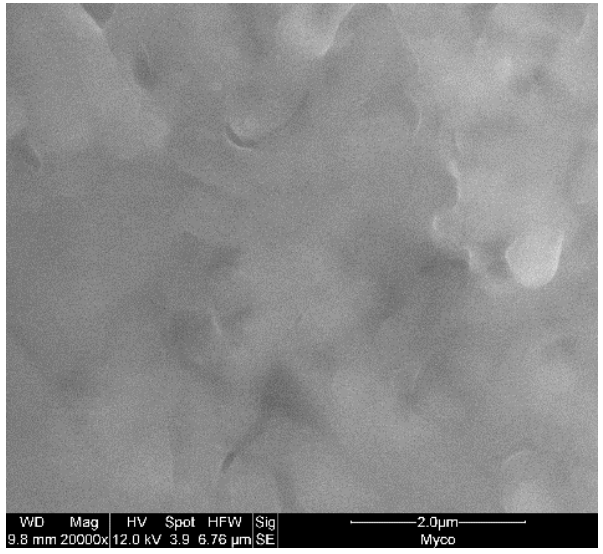
3D-printed centrifuge insert, “cone”



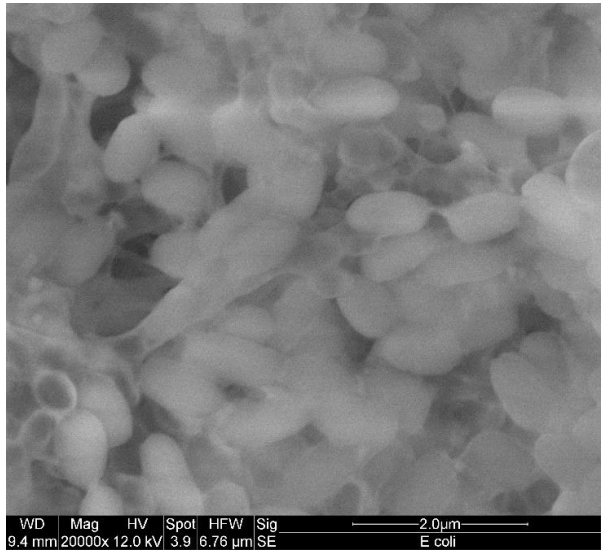
Scanning Electron Microscopy (SEM)



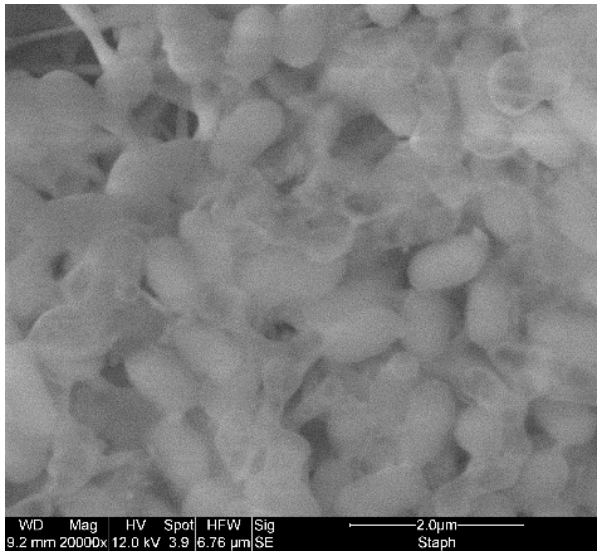
Nitrocellulose filter, no bacteria



M. smegmatis



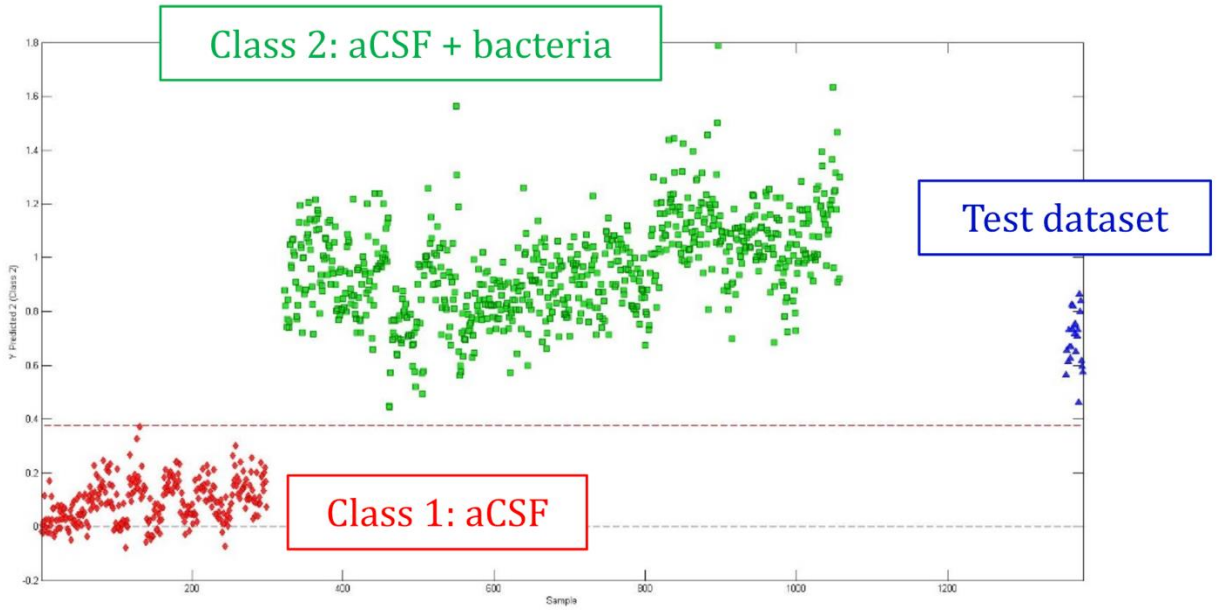
E. coli



S. aureus

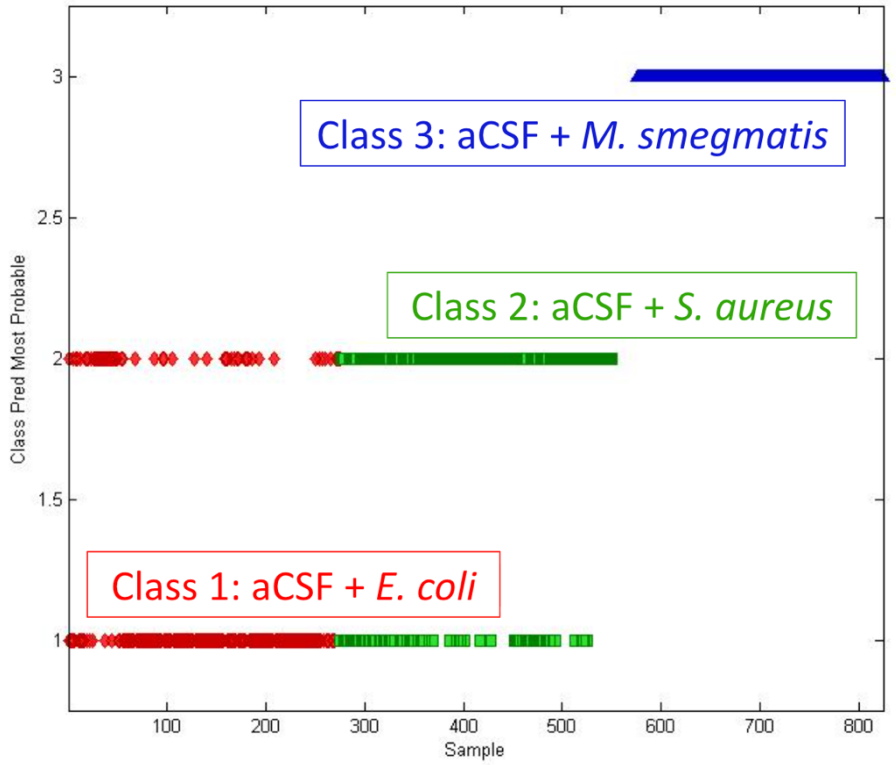
Two-class PLSDA

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



Three-class PLSDA

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



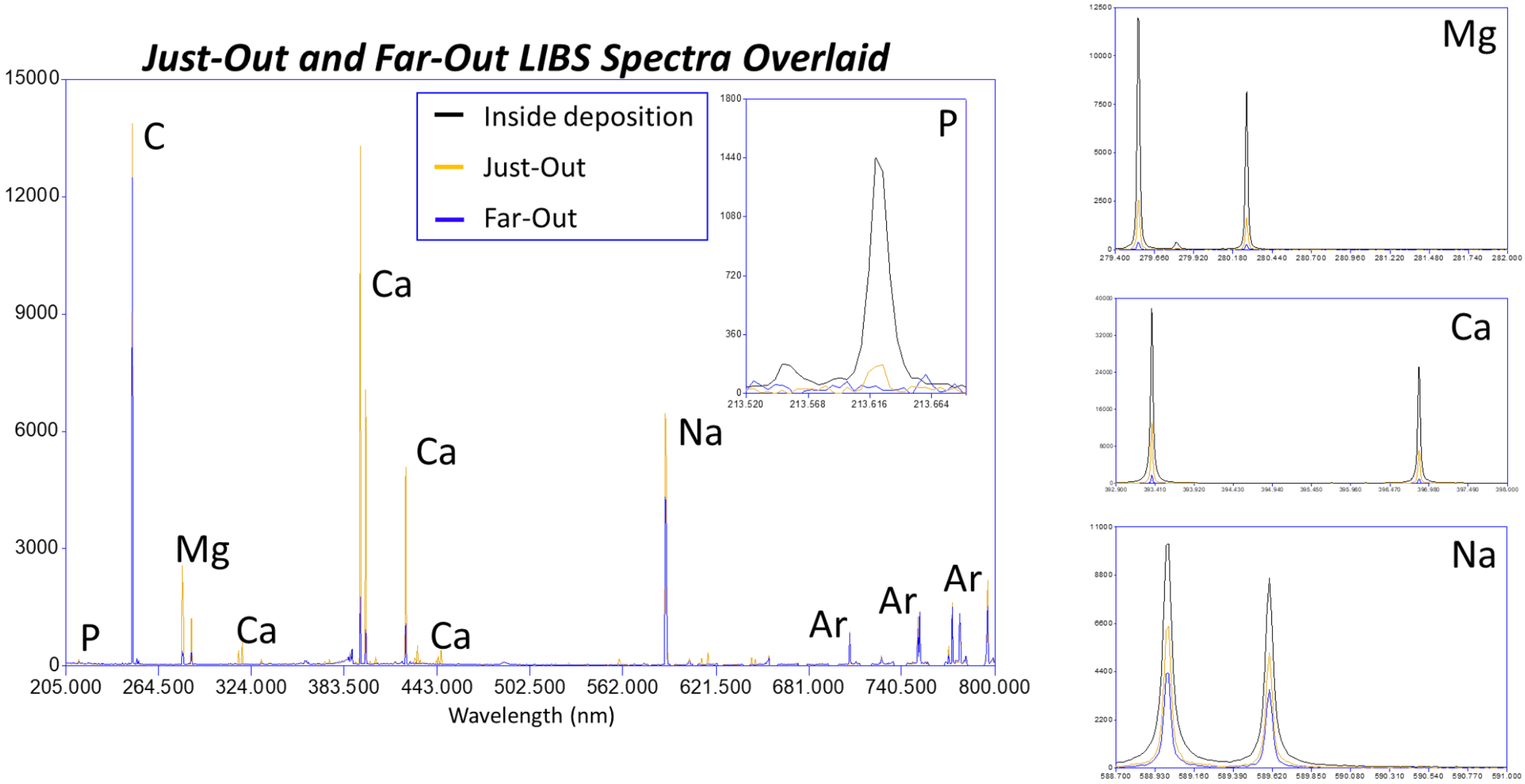
PCA-ANN with Full-Spectrum Analysis

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 %


Just-Out and Far-Out LIBS Spectra Overlaid
Inside deposition
Just-Out
Far-Out
C
Ca
Ca
Ca
Na
P
Mg
Ca
Ca
Ar
Ar
Ar
P
Mg
Ca
Na
Wavelength (nm)