LIBS for the Rapid Detection and Diagnosis of Pathogenic Bacteria in Human Clinical Specimens: Blood, Urine, and Cerebrospinal Fluid

Steven J. Rehse, Emma Blanchette, Emily Tracey, August Baughan, Grace Johnson, Hadia Malik, Caroline Aliontе, Isabella Arthur, Jasmine Saad, Rachel Chevalier, Abdullah Mustafa, Lauren Dmytrow, Mila Vasquez, Nicholas Bolton, Matteo Pontoni

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Historical Notes

LIBS has been used to detect/identify bacteria since 2003.

Detection of bacteria by time-resolved laser-induced breakdown spectroscopy

Stéphane Morel, Nicolas Leone, Philippe Adam, and Jacques Amouroux

6184 APPLIED OPTICS / Vol. 42, No. 30 / 20 October 2003

Bacillus globigii BG-1

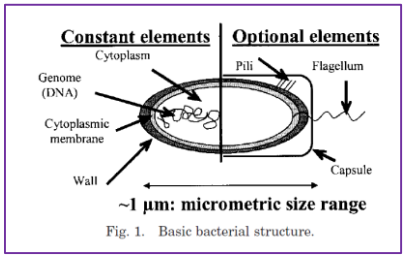

Fig. 1. Basic bacterial structure.

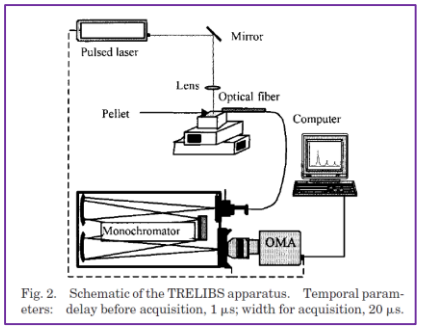

Fig. 2. Schematic of the TRELIBS apparatus. Temporal parameters: delay before acquisition, 1 µs; width for acquisition, 20 µs.

Laser-Induced Breakdown Spectroscopy Detection and Classification of Biological Aerosols*

JOHN D. HYBL, GREGG A. LITHGOW, and STEVEN G. BUCKLEY†

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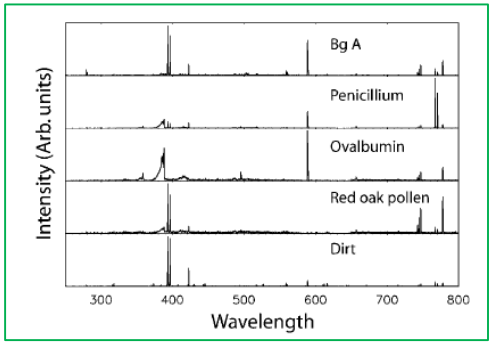
Volume 57, Number 10, 2003 APPLIED SPECTROSCOPY 1207



Bacillus subtilis var. niger

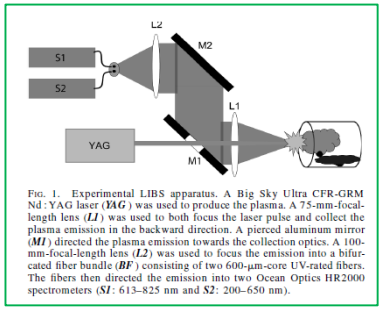


FIG. 1. Experimental LIBS apparatus. A Big Sky Ultra CFR-GRM Nd:YAG laser (*YAG*) was used to produce the plasma. A 75-mm-focal-length lens (*L1*) was used to both focus the laser pulse and collect the plasma emission in the backward direction. A pierced aluminum mirror (*M1*) directed the plasma emission towards the collection optics. A 100-mm-focal-length lens (*L2*) was used to focus the emission into a bifurcated fiber bundle (*BF*) consisting of two 600-µm-core UV-rated fibers. The fibers then directed the emission into two Ocean Optics HR2000 spectrometers (*S1*: 613–825 nm and *S2*: 200–650 nm).

Laser-induced breakdown spectroscopy of bacterial spores, molds, pollens, and protein: initial studies of discrimination potential

Alan C. Samuels, Frank C. DeLucia, Jr., Kevin L. McNesby, and Andrzej W. Miziolek

20 October 2003 / Vol. 42, No. 30 / APPLIED OPTICS 6205

Bacillus thuringiensis subsp. *kurstaki*, *Bacillus subtilis* (also known as *Bacillus globigii*) subsp. *niger*, and *Bacillus cereus* 6E1

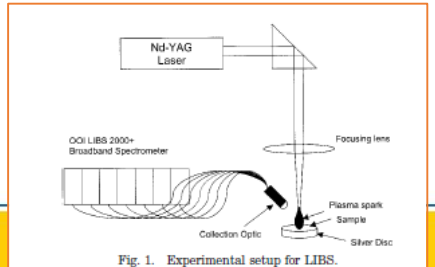
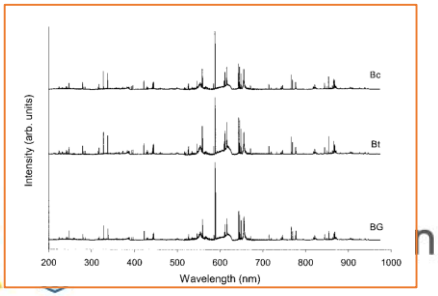


Fig. 1. Experimental setup for LIBS.



ndsor

Historical Notes

Significant achievements followed quickly.

Anal. Chem. **2005,** *77,* 631−638

Feasibility of Detection and Identification of Individual Bioaerosols Using Laser-Induced Breakdown Spectroscopy

P. B. Dixon and D. W. Hahn*

Department of Mechanical and Aerospace Engineering, University of Florida, Gainesville, Florida 32611-6300

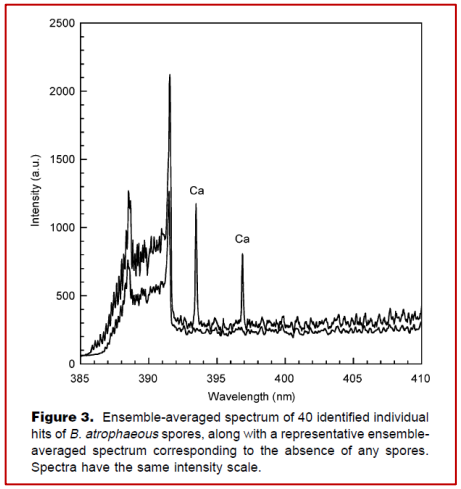


Figure 3. Ensemble-averaged spectrum of 40 identified individual hits of *B. atrophaeous* spores, along with a representative ensemble-averaged spectrum corresponding to the absence of any spores. Spectra have the same intensity scale.

Detection of single *Bacillus* spores

JOURNAL OF APPLIED PHYSICS **99**, 084701 (2006)

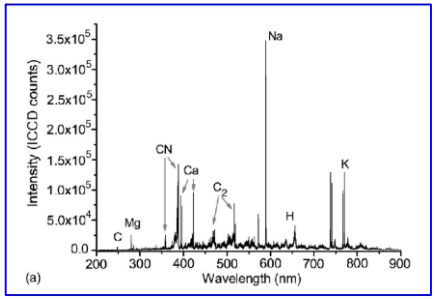
Femtosecond time-resolved laser-induced breakdown spectroscopy for detection and identification of bacteria: A comparison to the nanosecond regime

Matthieu Baudelet, Laurent Guyon, Jin Yu,[a] and Jean-Pierre Wolf
Laboratoire de Spectrométrie Ionique et Moléculaire, UMR CNRS 5579, Université Claude Bernard-Lyon 1, 43, Boulevard du 11 Novembre 1918, F-69622 Villeurbanne Cedex, France

Tanguy Amodeo and Emeric Fréjafon
Institut National de l'Environnement Industriel et des Risques (INERIS), Parc technologique ALATA, BP2, 60550 Verneuil-en-Halatte

Patrick Laloi
Laboratoire de Microbiologie et Génétique, UMR CNRS 5122, Université Claude Bernard-Lyon 1, 43, Boulevard du 11 Novembre 1918, F-69622 Villeurbanne Cedex, France

Escherichia coli and *Bacillus subtilis* with fs-LIBS



Discrimination of three strains of *Escherichia coli*, mold, and *Candida albicans* yeast

APPLIED PHYSICS LETTERS **90**, 163901 (2007)

***Escherichia coli* identification and strain discrimination using nanosecond laser-induced breakdown spectroscopy**

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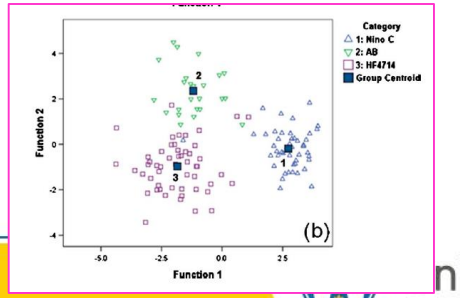
Sunil Palchaudhuri
Department of Immunology and Microbiology, Wayne State University, Detroit, Michigan 48201

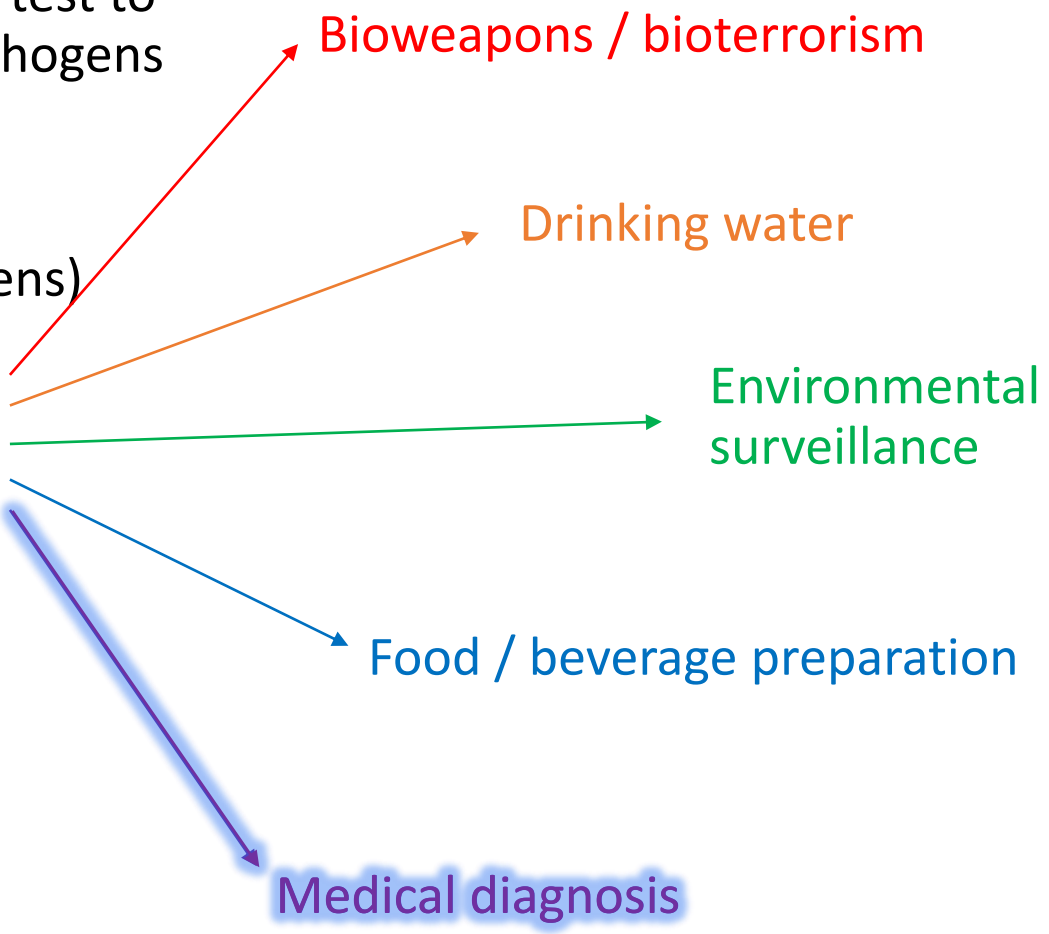
(Received 26 January 2007; accepted 15 March 2007; published online 16 April 2007)



The Reasons?

There did not exist at the time, ***and there still is not now***, a diagnostic test to detect and diagnose bacterial pathogens that can be:

- highly-flexible (multiple pathogens)
- very rapid (under 5 minutes)
- robust (field portable)
- relatively simple (minimal prep, performed by a non-expert)
- few to no consumables (biodegradation, cost)
- sensitive
- specific



To the present

Since then, progress has been extensive on:

- the number / variety of species
- sample preparation methods
- hardware setups
- chemometric algorithms
- number of bacteria detectable
- machine learning

To the present

Table 2
A summary of all the bacterial species/strains tested with LIBS.

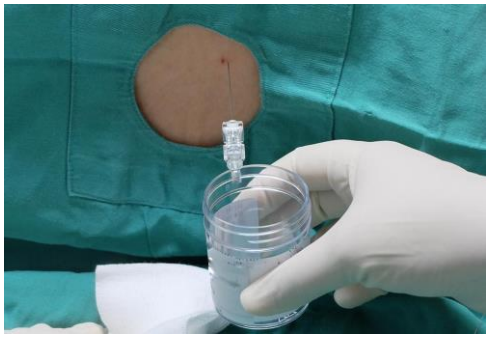
Micro-organism	Reference	Form	Chemometric utilized	Laser wavelength[a]
Acinetobacter baumannii ATCC BAA-1789	[66]	Colony on blood agar	PCA/PLS1	1064
Acinetobacter baylyi	[48]	Pellet, freeze-dried powder	Hyperspace projection of trace elements	810 (fs)
Acinetobacter calcoaceticus [FJ816073][b]	[71]	Colony on glass slide	PCA/PLS-RA	800 (fs)
Arhodomonas sp. [EU308280]	[71]	Colony on glass slide	PCA/PLS-RA	800 (fs)
Bacillus anthracis var. Sterne	[44]	Thin lawn[c] on nylon filter	None	1064
Bacillus anthracis var. Sterne	[38]	Thin lawn on agar, glass slide	PCA/PLS1	1064
Bacillus atrophaeous	[41]	Spore, aerosol stream	None	1064
Bacillus atrophaeous	[52,37]	Dried film on Al disk, steel disk, polycarbonate disk	NN, MLSRA,PLS-DA	1064
Bacillus atrophaeous	[57]	Pellet, freeze-dried powder	SVM	1064
Bacillus aureus	[35]	Spore, EDB trap	None	355
Bacillus cereus 6E1	[29,32]	Thin lawn on silver membrane filter	PCA, linear correlation, and SIMCA	1064
Bacillus cereus ATCC 14603	[57]	Pellet, freeze-dried powder	SVM	1064
Bacillus globigii[d] BG-1	[39,40]	Pellet, freeze-dried powder	None	1064
Bacillus globigii BG-1	[40]	Spore, aerosol stream	None	1064
Bacillus globigii BG-2	[39,40]	Pellet, freeze-dried powder	None	1064
Bacillus globigii BG-2	[40]	Spore, aerosol stream	None	1064
Bacillus globigii var. niger	[29,42,32]	Thin lawn on silver membrane filter	PCA, linear correlation, and SIMCA	1064
Bacillus globigii var. niger	[30]	Continually refreshed dense aerosol cloud (from powder) and aerosol stream	PCA	1064
Bacillus globigii var. niger	[35,68]	Powder on double-sided sticky tape	No, linear correlation, PCA, PLS-DA	1064 × 2 (DP)
Bacillus globigii 168	[43]	Colony (wet) on LB medium	None	532
Bacillus globigii	[47]	Thin film lawn on cellulose nitrate membrane filter	None	810 (fs), 1064
Bacillus globigii	[48]	Pellet, freeze-dried powder	Hyperspace projection of trace elements	810 (fs)
Bacillus globigii	[31]	Dried powder on solid substrate	PCA, HCA, PCA + LDA	1064
Bacillus globigii ATCC 23857	[66]	Colony on blood agar	PCA/PLS1	1064
Bacillus megaterium QM B1551	[43]	Colony (wet) on LB medium	None	532
Bacillus megaterium PV361	[43]	Colony (wet) on LB medium	None	532
Bacillus stearothermophilus ATCC 12979	[57]	Pellet, freeze-dried powder	SVM	1064
Bacillus thurengensis	[39,40]	Pellet, freeze-dried powder	None	1064
Bacillus thurengensis var. kurstaki	[29,32]	Thin lawn on silver membrane filter	PCA, linear correlation, and SIMCA	1064
Bacillus thurengensis var. kurstaki	[44]	Thin lawn on nylon filter	None	1064
Bacillus thurengensis T34	[43]	Colony (wet) on LB medium	None	532
Bacillus thuringiensis ATCC 51912	[57]	Pellet, freeze-dried powder	SVM	1064
Bacillus sp. [GQ392044]	[71]	Colony on glass slide	PCA/PLS-RA	800 (fs)
Bacillus sp. [GQ226038]	[71]	Colony on glass slide	PCA/PLS-RA	800 (fs)
Bacillus sp. [HM026606]	[71]	Colony on glass slide	PCA/PLS-RA	800 (fs)
Enterobacter cloacae [FJ194527]	[71]	Colony on glass slide	PCA/PLS-RA	800 (fs)
Enterobacter cloacae ATCC 13047	[64,67]	Thin lawn on nutrient-free agar	DFA, PLS-DA	1064
Enterobacter sp. [CP000653]	[71]	Colony on glass slide	PCA/PLS-RA	800 (fs)
Enterobacter sp. [GU586319]	[71]	Colony on glass slide	PCA/PLS-RA	800 (fs)

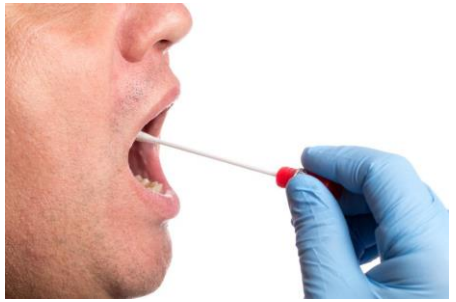
Specimen presentation

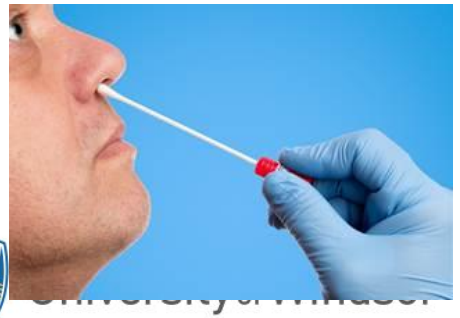
In my research, I am almost completely focused on developing a realistic clinical testing methodology.

Current methods of LIBS bacterial analysis are completely inadequate for this purpose.

Most samples are obtained as fluid specimens, need to perform LIBS on <u>that</u> to be truly rapid.







Outline for rest of talk

1. Explain how we perform LIBS on bacteria in my lab in general

2. Three case studies

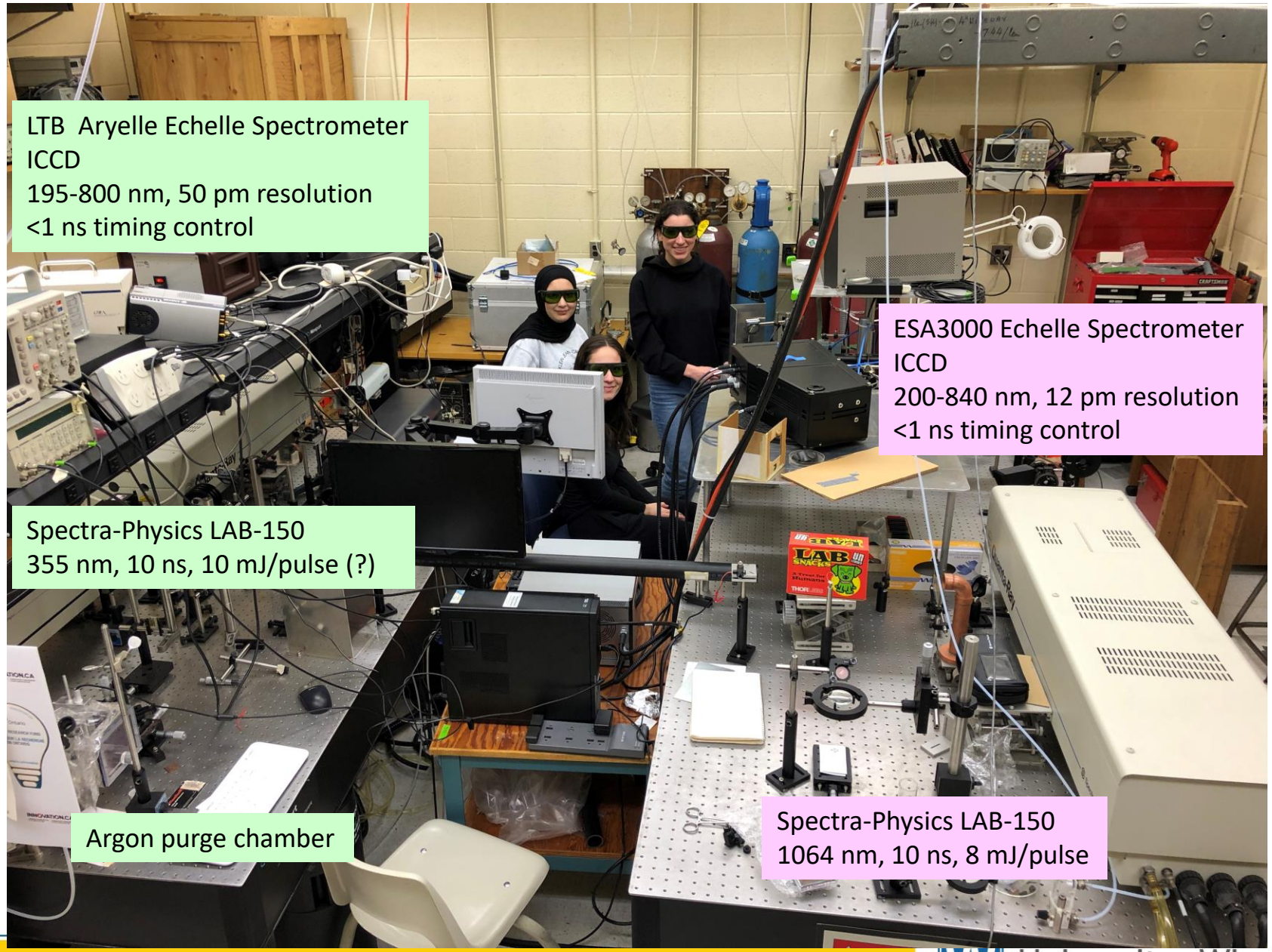
    2a. Using the same sample preparation and data analysis

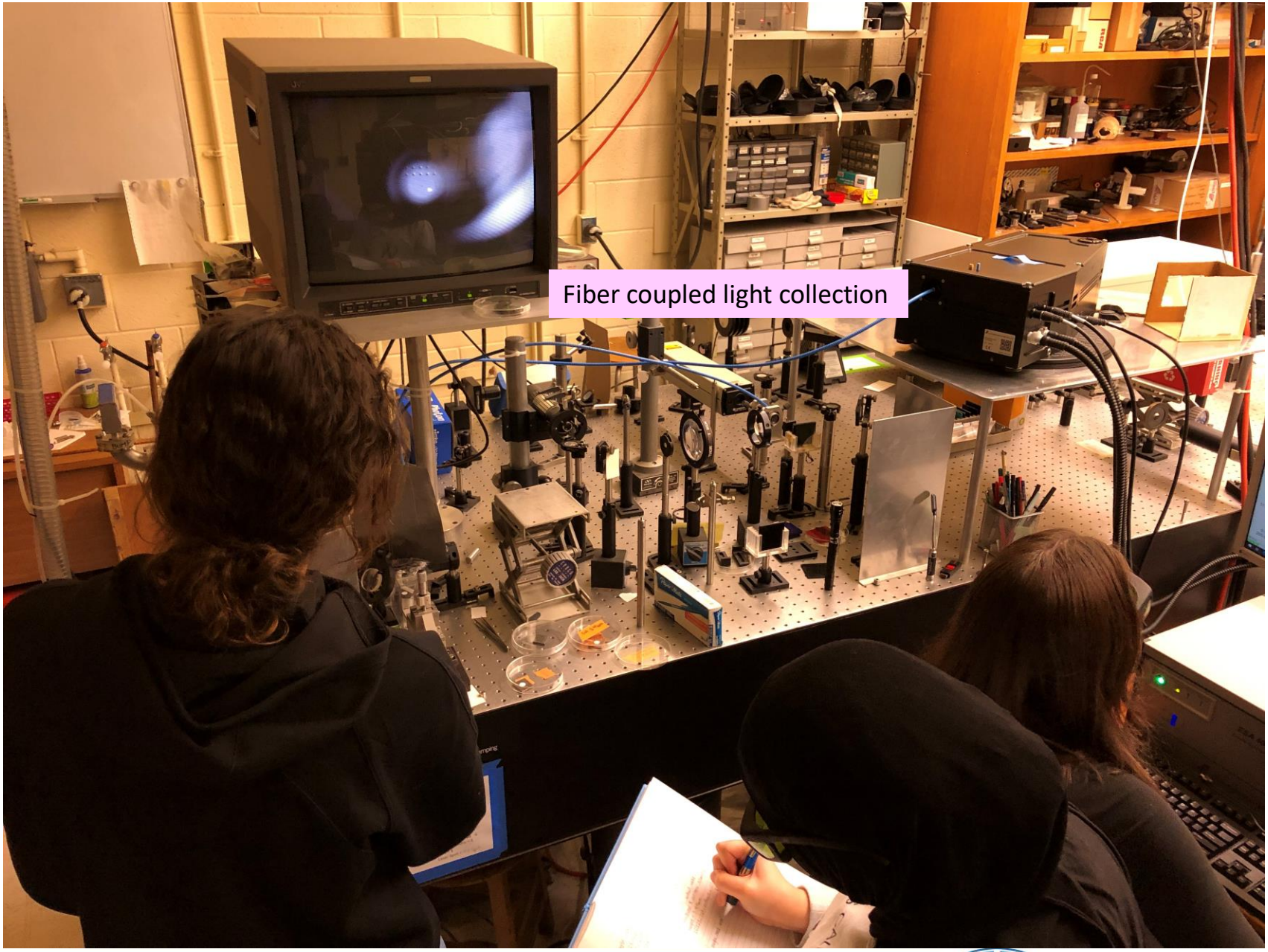
        - **Blood**

        - Urine

    2b. Using a modified sample preparation and data analysis

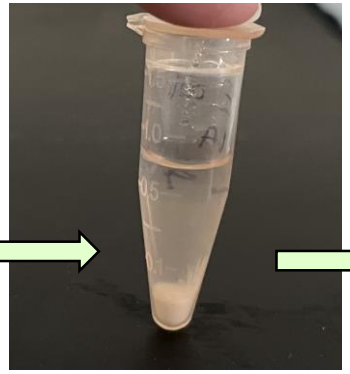
        - Cerebrospinal fluid


LTB Aryelle Echelle Spectrometer
ICCD
195-800 nm, 50 pm resolution
<1 ns timing control
ESA3000 Echelle Spectrometer
ICCD
200-840 nm, 12 pm resolution
<1 ns timing control
Spectra-Physics LAB-150
355 nm, 10 ns, 10 mJ/pulse (?)
Argon purge chamber
Spectra-Physics LAB-150
1064 nm, 10 ns, 8 mJ/pulse


Fiber coupled light collection

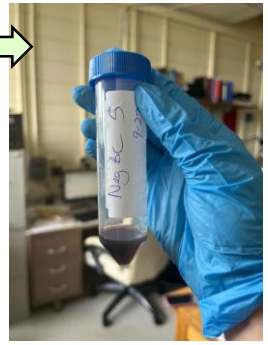
Methodology – Bacterial Growth & Sample Prep

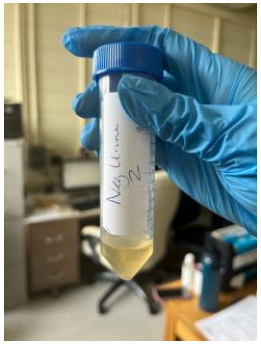
Bacteria cultured on agar plate



Harvested bacteria stored in $M\Omega H_2O$

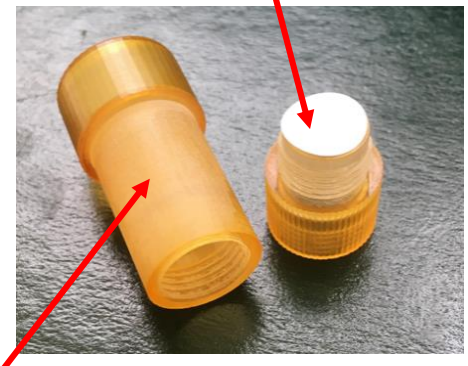
Bacteria-negative specimens collected from hospital



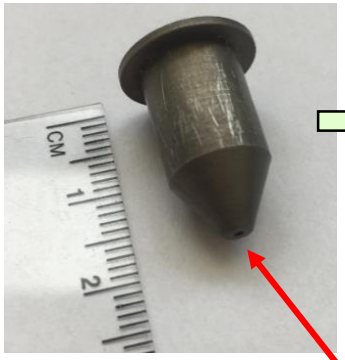


100 µL fluid specimen + 100 µL bacterial suspension pipetted into reassembled apparatus and sealed in centrifuge tube

9.5 mm diameter disposable filter



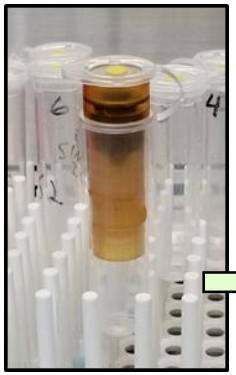
Disassembled 3D printed centrifuge insert



Aluminum cone with 1 mm diameter aperture at apex

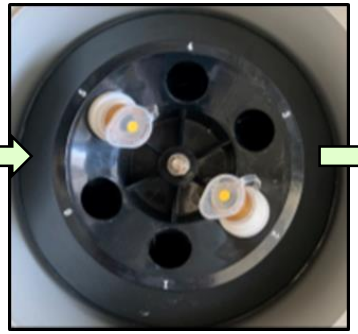
Centrifuge insert assembled with filter & cone

Methodology – Bacterial Growth & Sample Prep

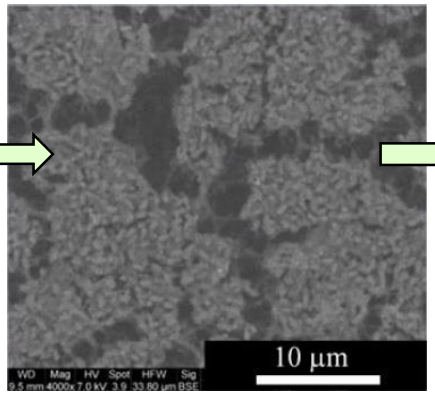


Assembled centrifuge tube insert

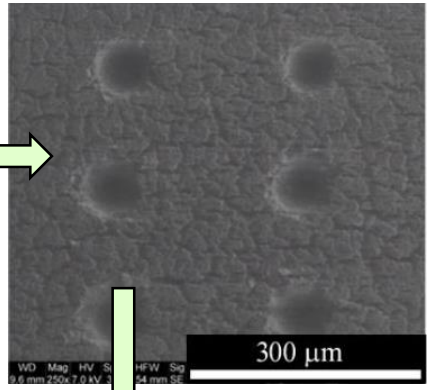
Samples centrifuged at 5000 RPM for five minutes



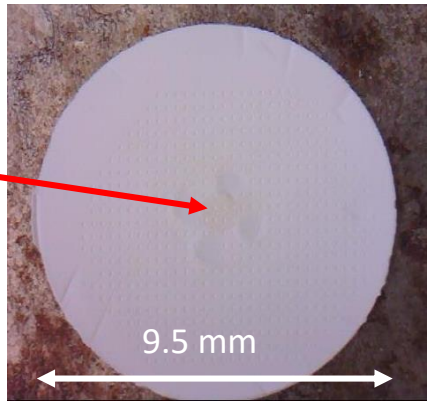
Insert disassembled, filter mounted on steel with double-sided sticky tape

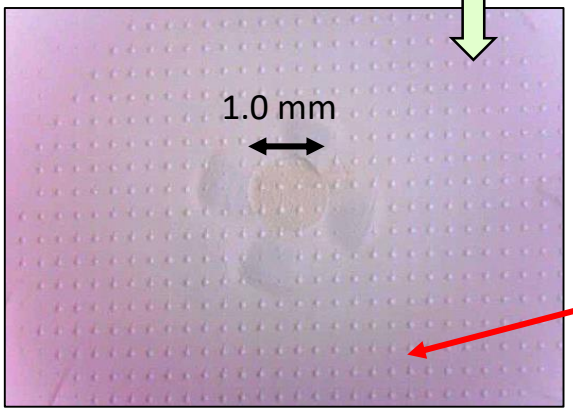


Sample ablated in Ar environment



Filter after centrifugation; bacteria concentrated at the center. We obtain 25-30 LIBS spectra per deposition (could be more)





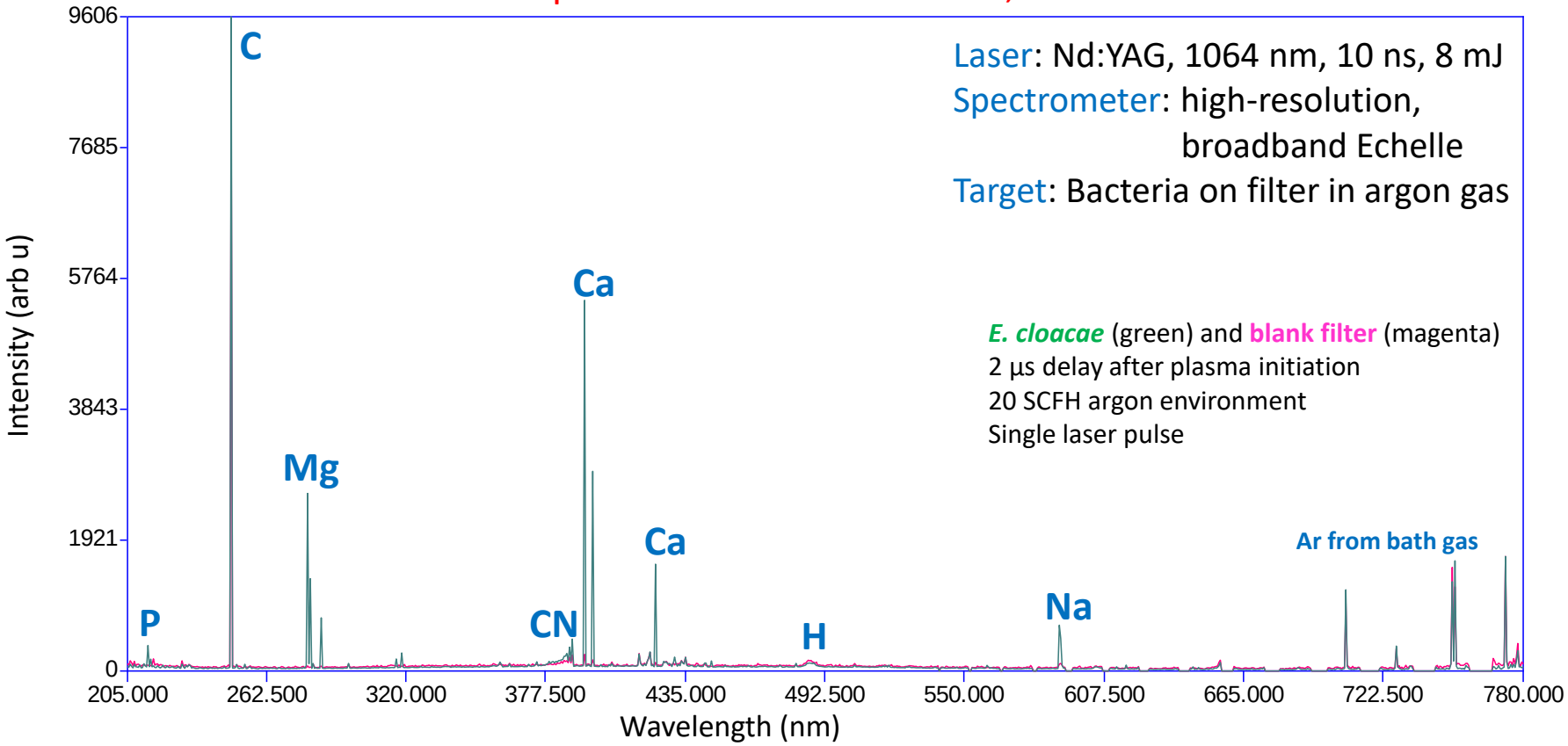
Magnification of the filter with a rastered array of laser ablation sites

Images of filter after data acquisition

Spectrum From Bacteria

An elemental assay of the bacterial cell composition!

Each spectrum obtained from 11,000 cells



Suggests a real-time method for pathogenic bacterial diagnosis.

Detecting Species in Fluids with Chemometrics

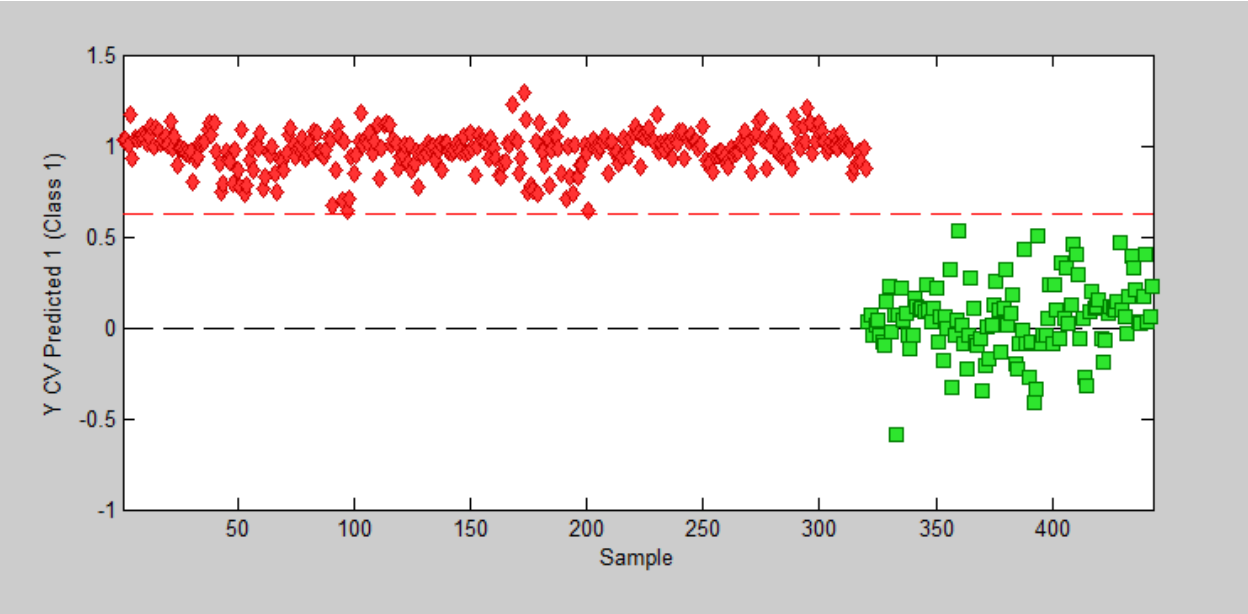
"Ratio Model" consists of:

- 15 emission line intensities from P, C, Mg, Ca, and Na and 92 simple ratios
- 107 total independent variables

PLS-DA on Ratio Model

PLS-DA implemented with PLS_Toolbox (Eigenvector, Inc)

- Run on desktop PC, takes seconds
- Always perform a two-class test and remove entire filters at a time for testing

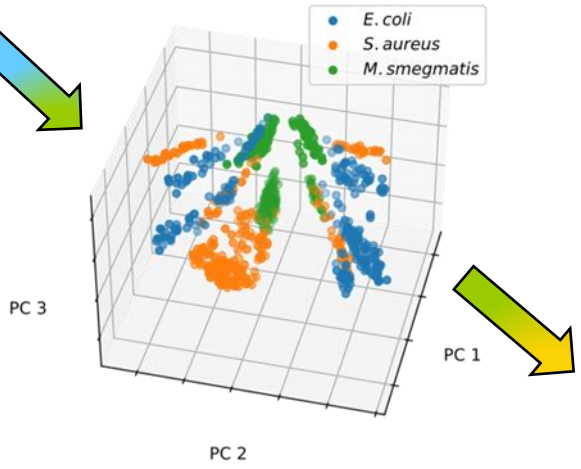


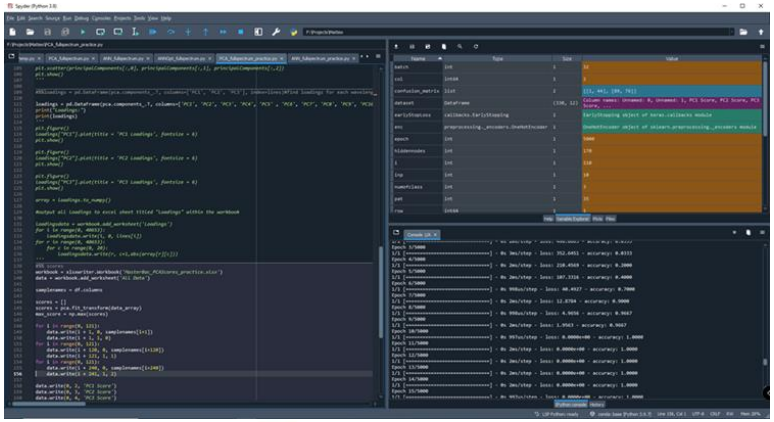
Diagnosing Species in Fluids with Machine Learning

- Started using the **"whole spectrum"** from 200 nm - 590 nm. 42,000 variables.
- Perform unsupervised PCA first (implemented in Python), reduce to 10 PC's.
- ANN models implemented in Python are trained on 80% of single shot data, 20% reserved for testing. (~15 seconds).
- ANN models/parameters are optimized on our data

PCA-ANN on Full Spectrum Data*

*The full spectrum spans 200 nm – 760 nm, but no lines of interest > 590 nm





Blood (sepsis)

Sepsis (bacterial blood infection) is a highly dangerous infection and is a common nosocomial (hospital acquired) infection.

Sepsis is not always caused by bacterial infection, but many cases are bacterial in origin.

A blood culture is required for the diagnosis of sepsis, but not all blood cultures will produce a positive test result.

Antibiotics are the preferred treatment for sepsis, but even with the best treatment the mortality for patients who have reached septic shock is no better than 50%.

The time it takes to initiate effective antimicrobial therapy is the single strongest predictor of patient outcome, with every hour of delay increasing patient mortality.

E. Whitnack, Sepsis, in: N.C. Engleberg, V.J. DiRita, T. Dermody, M. Schaechter (Eds.), Mechanisms of Microbial Disease, 3rd ed, Lippincott Williams & Wilkins, 2007, pp. 564–572.

A. Kumar, D. Roberts, K.E. Wood, B. Light, J.E. Parrillo, et al., Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock, Crit. Care Med. 34 (2006) 1589–1596.

Blood (sepsis)

The standard diagnostic blood cultures are slow and labor intensive, usually requiring a two-step procedure:

1) an initial culture taking up to 120 h

2) a 24-48 h subculture to identify the pathogens detected in a positive initial culture.

Automated blood culture system

Culturing and diagnosis done manually

J.C. Lagier, S. Edouard, I. Pagnier, O. Mediannikov, M. Drancourt, D. Raoult, Current and past strategies for bacterial culture in clinical microbiology, Clin. Microbiol. Rev. 28 (2015) 208–236, https://doi.org/10.1128/cmr.00110-14

D.J. Shin, N. Andini, K. Hsieh, S. Yang, T.-H. Wang, Emerging analytical techniques for rapid pathogen identification and susceptibility testing, Ann. Rev. Anal. Chem. 12 (2019) 41-67, https://doi.org/10.1146/annurev-anchem-061318-115529

Detecting Infection in Blood with a PLS-DA

Bacterial-negative specimens obtained from Windsor Regional Hospital with the anticoagulant sodium polyanetholesulfonate (SPS) present.

Five different blood samples from different patients were provided to account for the difference between patient physiology.

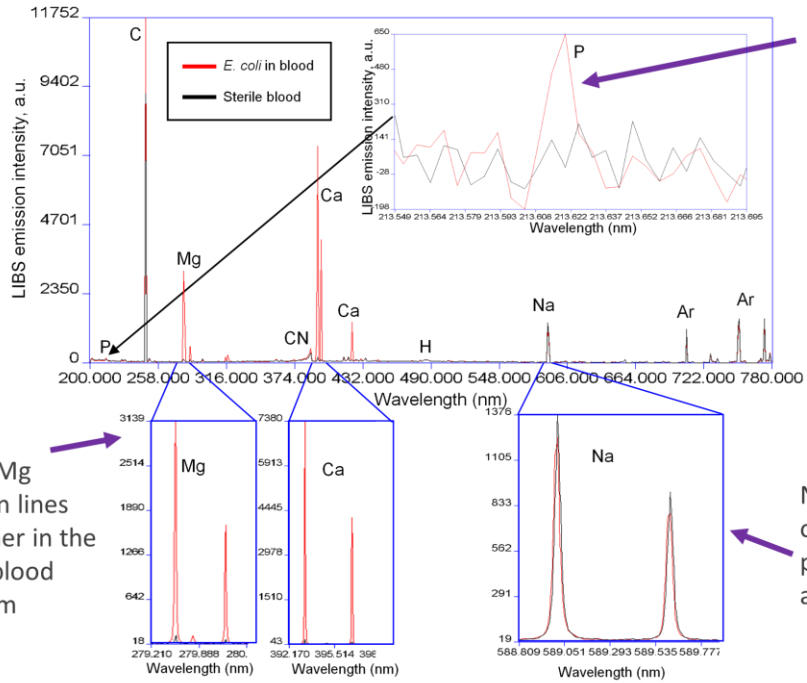
Specimens were vortex-mixed prior to handling to insure the redistribution of any suspended particles.

DETECTION IN BLOOD (individual spectra)

96.3% sensitivity – 600 bacterial spectra
98.6% specificity – 206 blood spectra
11,000 cells / spectrum

DETECTION IN BLOOD (spectra added)

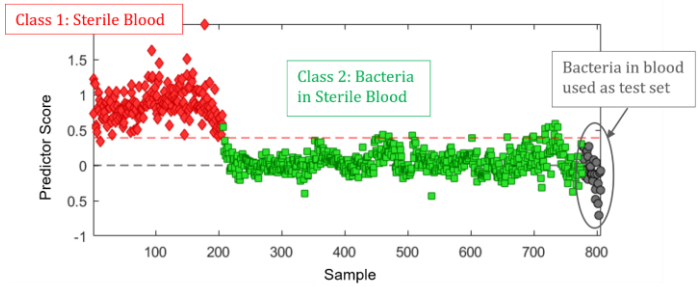
19 of 19 filters positive, 100% sensitivity
7 of 7 filters negative, 100% specificity



P line is small in the spiked blood, but not present in the sterile blood

Ca and Mg emission lines are higher in the spiked blood spectrum

Na emission observed is partially due to anticoagulant



Prepared Samples:

- 5 filters each of *S. aureus*, *E. coli*, *E. cloacae*
- 4 filters of *P. aeruginosa*
- 7 filters of sterile blood
- 30 LIBS laser shots from each deposition

Diagnosing Infection in Blood with PCA-ANN

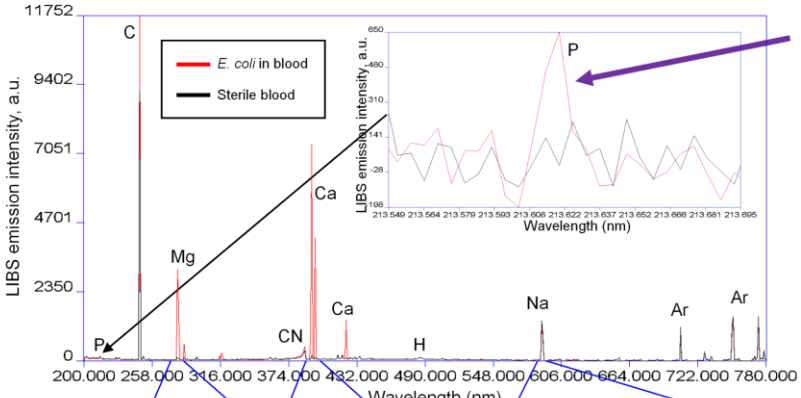
Bacterial-negative specimens obtained from Windsor Regional Hospital with the anticoagulant sodium polyanetholesulfonate (SPS) present.

Five different blood samples from different patients were provided to account for the difference between patient physiology.

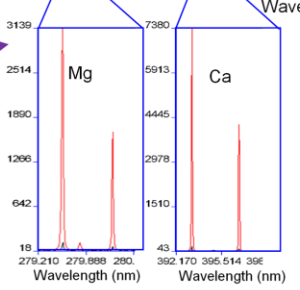
Specimens were vortex-mixed prior to handling to insure the redistribution of any suspended particles.

PCA-ANN With Full Spectrum Data*				
	S. aureus	*E. coli*	*E. cloacae*	*P. aeruginosa*
Sensitivity	100 %	100 %	100 %	100 %
Specificity	100 %	100 %	100 %	100 %
Classification Error	0.00 %	0.00 %	0.00 %	0.00 %

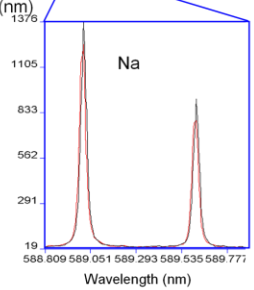
*classification using 80:20 split



P line is small in the spiked blood, but not present in the sterile blood



Ca and Mg emission lines are higher in the spiked blood spectrum



Na emission observed is partially due to anticoagulant

Prepared Samples:

- 5 filters each of *S. aureus*, *E. coli*, *E. cloacae*
- 4 filters of *P. aeruginosa*
- 7 filters of sterile blood
- 30 LIBS laser shots from each deposition

Urine (UTI)

UTI (urinary tract infections) are one of the most common infections in adult women, with up to 50% of women having experienced at least one UTI in their lifetime and upwards of 10% of women experiencing at least one UTI annually.

In 2007, UTI resulted in approximately 8.6 million health care visits in the United States with an estimated cost of 1.6 billion US dollars.

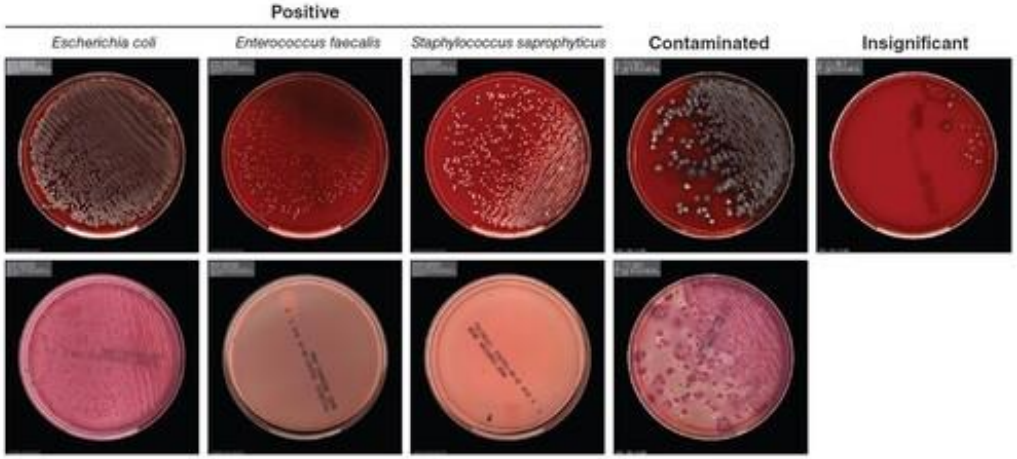
In addition to the prevalence in the population, UTI are also the most common nosocomial infection, with up to 80% of hospital-acquired UTI associated with the use of a bladder catheter.

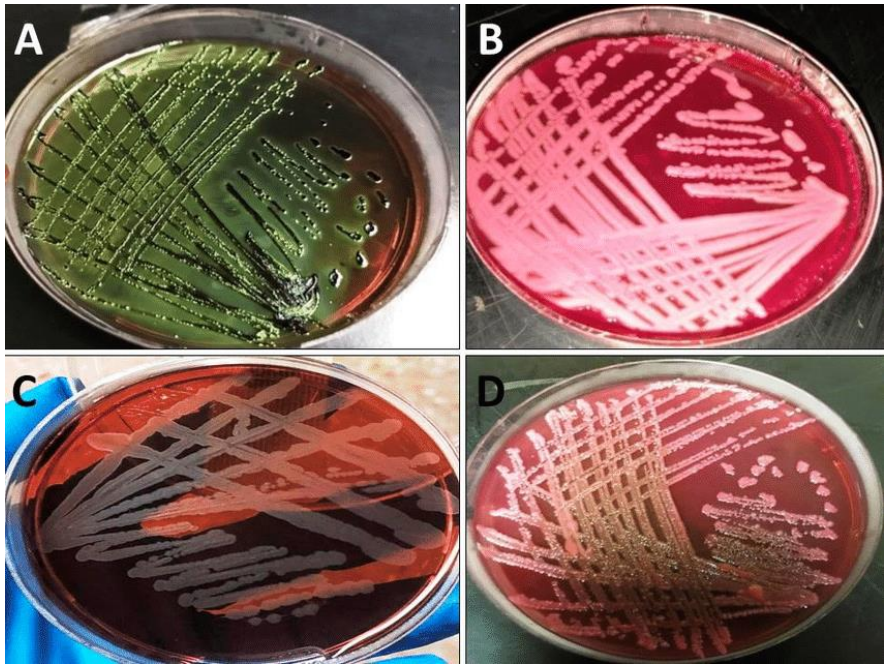
Even a single catheterization can lead to UTI due to contamination of the catheter tip.

T.K. Price, E.E. Hilt, T.J. Dune, E.R. Mueller, A.J. Wolfe, L. Brubaker, Urine trouble: should we think differently about UTI?, Int. Urogynecol. J. 29 (2018) 205-210,
S.M. Schappert, E.A. Rechtsteiner, Ambulatory medical care utilization estimates for 2007, Vital Health Stat. 13 169 (2011) 1-38, PMID: 21614897.

J.M.T. Barfor, K. Anson, Y.Hu, A.R.M. Coates, A model of catheter-associated urinary tract infection initiated by bacterial contamination of the catheter tip, BJU Int. 102 (2008) 67-74.

Urine (UTI)





https://www.clinmicronow.org/doi/10.1128/9781683670438.CMPH.ch3.11-1
https://www.congress-intercultural.eu/it/?i=urine-good-hands-diagnosing-utis-with-urine-cultures-mm-vV1rkGCV

Detecting Infection in Urine with a PLS-DA

Bacterial-negative urine specimens obtained from Windsor Regional Hospital.

Urine specimens from four different patients were characterized to account for differences in patient physiology.

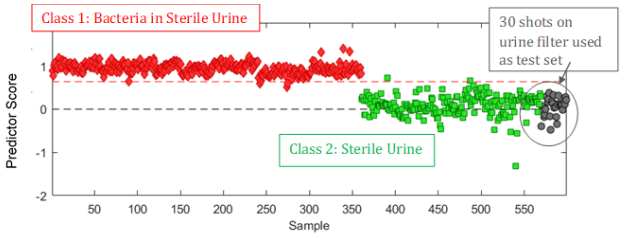
Specimens were vortex-mixed prior to handling to insure the redistribution of any suspended particles.

DETECTION IN URINE (individual spectra)

98.9% sensitivity – 360 bacterial spectra
100% specificity – 240 urine spectra
11,000 cells per spectrum

DETECTION IN URINE (spectra added)

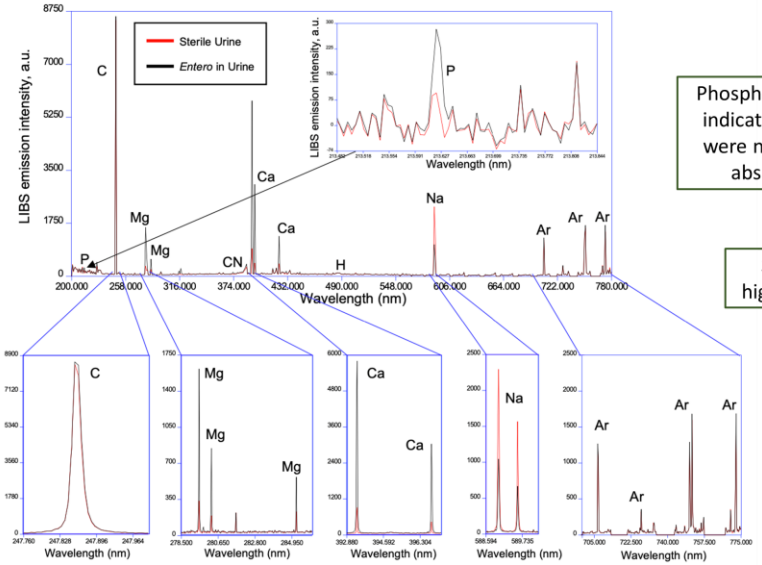
12 of 12 filters positive, 100% sensitivity
8 of 8 filters negative, 100% specificity



Strong carbon emission is predominantly from the nitrocellulose filter

Magnesium lines are greater in spiked urine, an indication of bacteria

Calcium lines higher in spiked urine samples, likely due to bacteria



Phosphorus lines are highly indicative of bacteria, and were never present in the absence of bacteria

Sodium emission higher in sterile urine

Argon lines are from the chamber environment where the samples are shot in

Prepared Samples:

- 4 filters each of *S. aureus*, *E. coli*, *E. cloacae*
- 8 filters of sterile urine
- 30 LIBS laser shots from each deposition

Diagnosing Infection in Urine with PCA-ANN

Bacterial-negative urine specimens obtained from Windsor Regional Hospital.

Urine specimens from four different patients were characterized to account for differences in patient physiology.

Specimens were vortex-mixed prior to handling to insure the redistribution of any suspended particles.

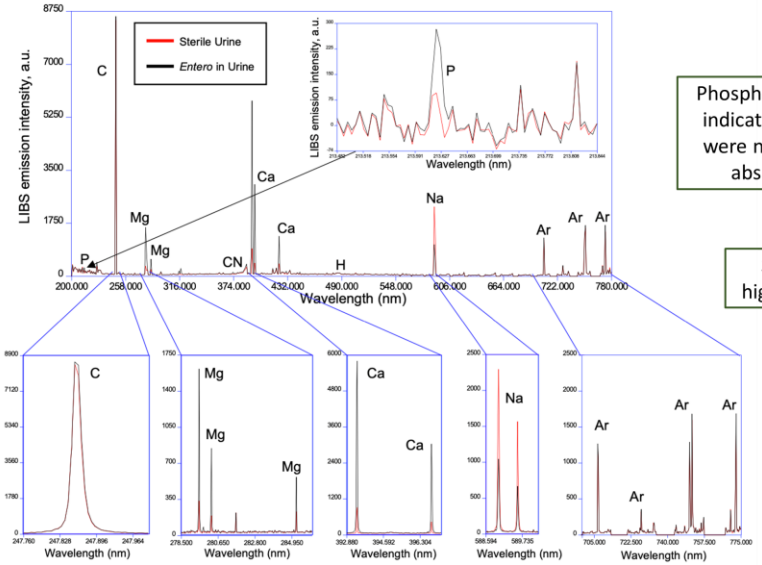
PCA-ANN With Full Spectrum Data*			
	S. aureus	*E. coli*	*E. cloacae*
Sensitivity	100 %	100 %	91.67 %
Specificity	100 %	95.83 %	100 %
Classification Error	0.00 %	2.09 %	4.17 %

*classification using 80:20 split

Strong carbon emission is predominantly from the nitrocellulose filter

Magnesium lines are greater in spiked urine, an indication of bacteria

Calcium lines higher in spiked urine samples, likely due to bacteria



Phosphorus lines are highly indicative of bacteria, and were never present in the absence of bacteria

Sodium emission higher in sterile urine

Argon lines are from the chamber environment where the samples are shot in

Prepared Samples:

- 4 filters each of *S. aureus*, *E. coli*, *E. cloacae*
- 8 filters of sterile urine
- 30 LIBS laser shots from each deposition

External Validation of PCA-ANN in Blood and Urine

- External validation done in both urine and blood (whole filters withheld)
- ANN model run 10 times per excluded filter
- Improvements need to be made on quality/consistency of spectra, not ML

average sensitivity of 74.6%

Urine	***E. coli***	***S. aureus***	***E. cloacae***	
Average Sensitivity	63.58 %	86.92 %	62.33 %	
Blood	***E. coli***	***S. aureus***	***E. cloacae***	***P. aeruginosa***
Average Sensitivity	93.73 %	61.73 %	91.60 %	95.0 %

average sensitivity of 85.5%

Urine

Sensitivity of each E. coli *filter removed from the model to be externally validated. ANN run 10 times per filter.*

E. coli	Predicted			
Sample #	*S. aureus*	*E. coli*	*E. cloacae*	Sensitivity
1	30	250	20	0.833
2	1	138	161	0.460
3	11	153	136	0.510
4	72	222	6	0.74
Sum	114	763	323	0.636

Blood

Sensitivity results for S. aureus *filters removed from the model to be externally validated. ANN run 10 times per filter.*

S. aureus	Predicted				
Sample #	*S. aureus*	*E. coli*	*E. cloacae*	*P. aeruginosa*	Sensitivity
1	50	54	86	110	0.167
2	300	0	0	0	1.000
3	1	27	266	6	0.033
4	290	10	0	0	0.967
5	285	13	0	2	0.950
Sum	926	104	352	118	0.617

Cerebrospinal Fluid (meningitis)

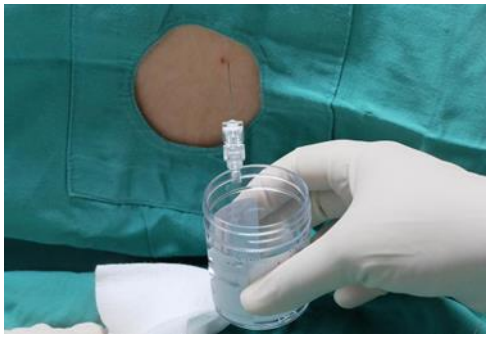
Cerebrospinal fluid (CSF) is the fluid the brain, cranium, and spinal cord are bathed in.

Meningitis is an infection of the brain and spinal cord's protective layers (cells in CSF).

Meningitis may be viral or bacterial in nature.

Delays and inappropriate antibiotic use can lead to severe complications, including irreversible brain damage or death.

The gold standard for diagnosis is a spinal tap (lumbar puncture) to remove **cerebrospinal fluid (CSF)** followed by culture, serology, and analysis.



Slane, Valori H. and Chandrashekhar G. Unakal. “Tuberculous Meningitis.” StatPearls, StatPearls Publishing, 18 November 2022.
Telano, Lauren N. and Stephen Baker. “Physiology, Cerebral Spinal Fluid.” StatPearls, StatPearls Publishing, 4 July 2023.

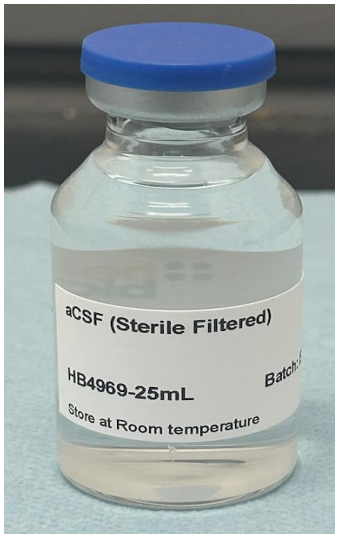
Cerebrospinal Fluid (meningitis)

Spinal taps are rarely performed in Windsor.

Artificial cerebrospinal fluid was used in this study (**aCSF**).

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

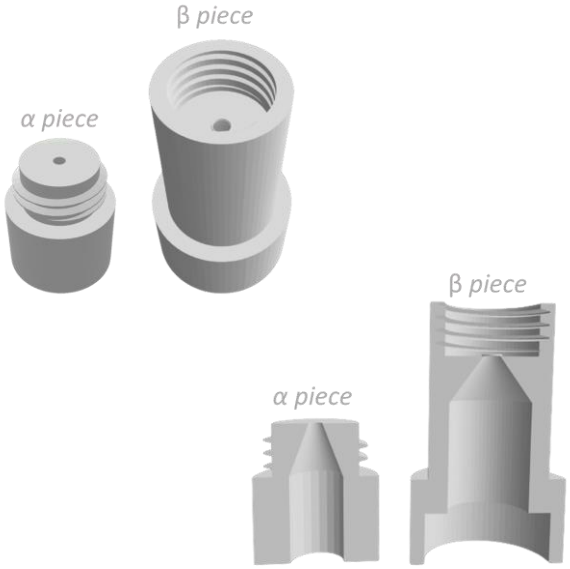


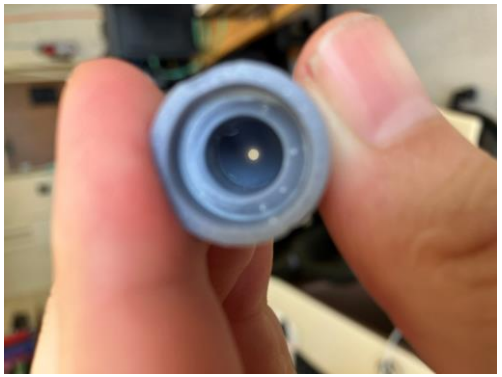
New Apparatus Design

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

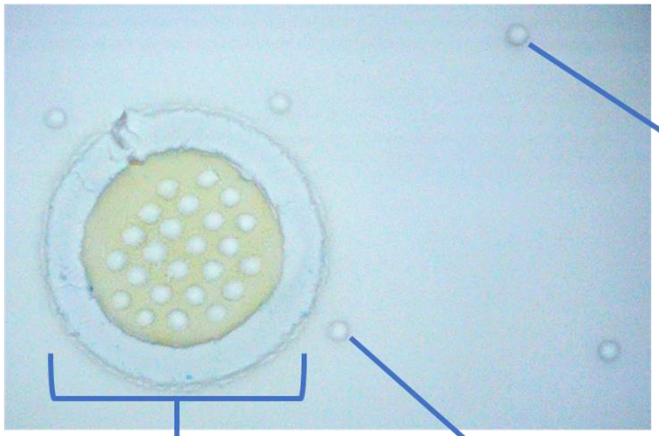
Stronger bacterial signal and more reproducible data





New Apparatus Data

nitrocellulose filter
(0.45 µm pore size)

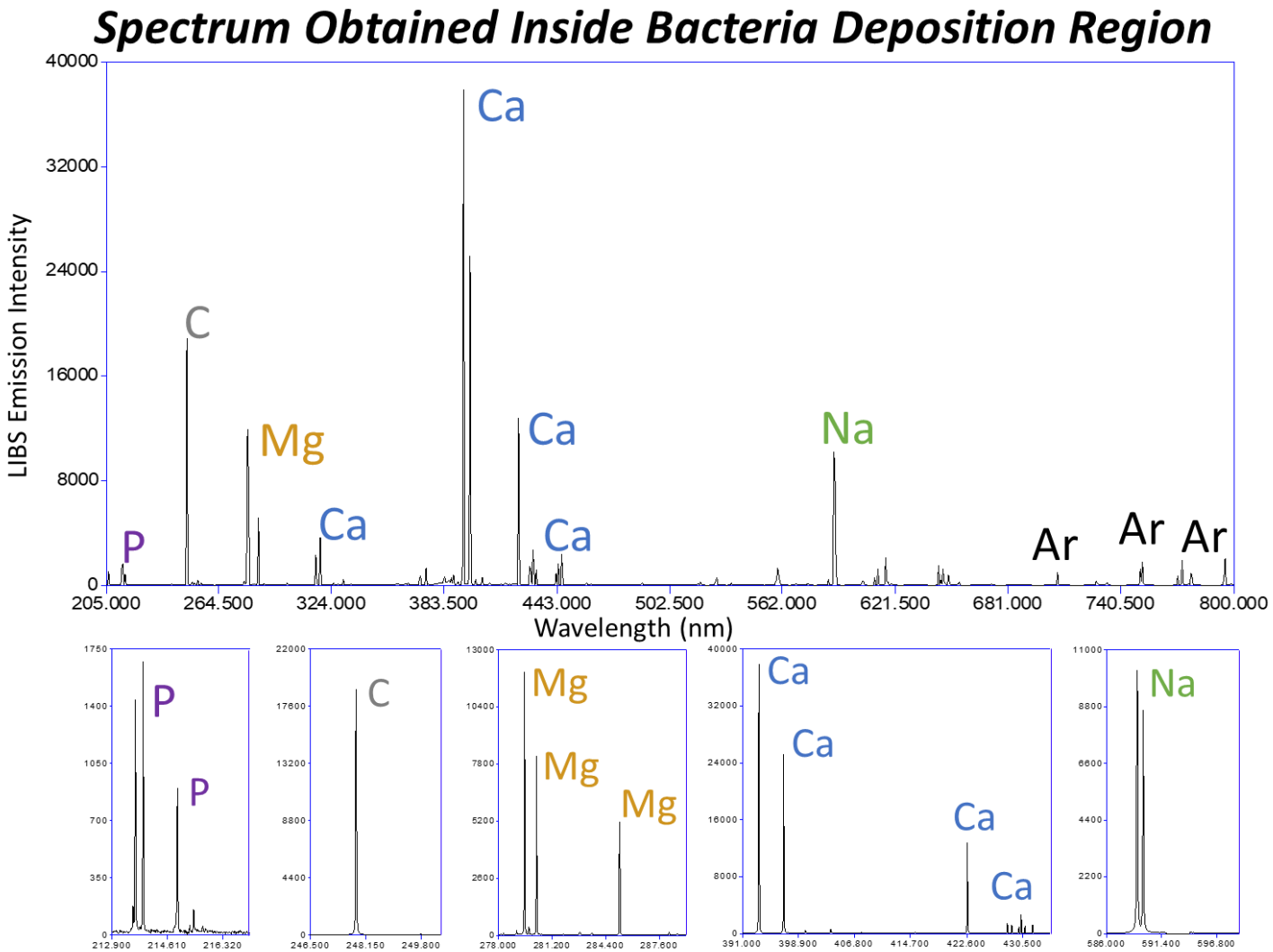


Laser shot obtained far from deposition region and near edge of filter or "Far-Out"

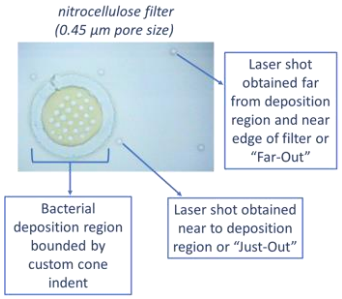
Bacterial deposition region bounded by custom cone indent

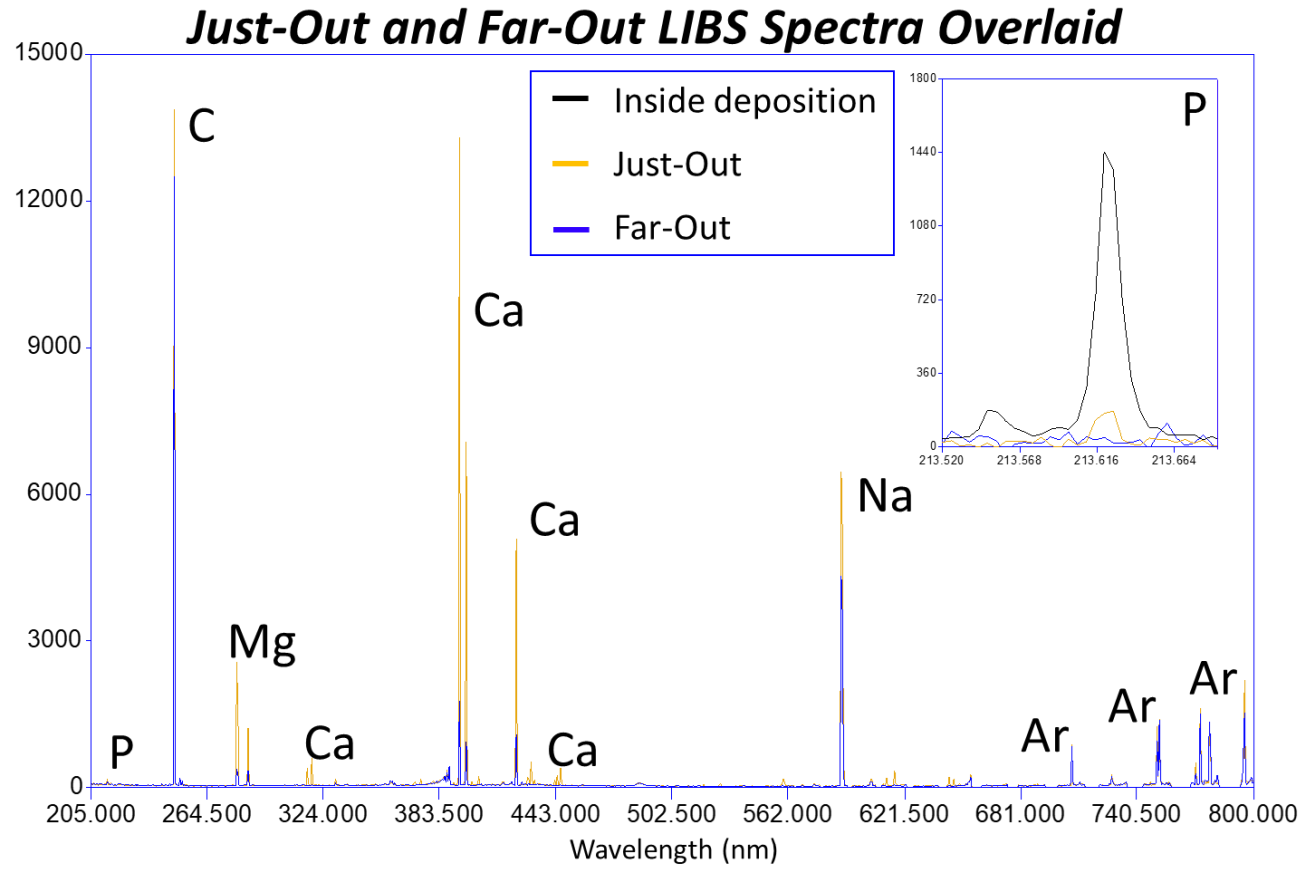
Laser shot obtained near to deposition region or "Just-Out"

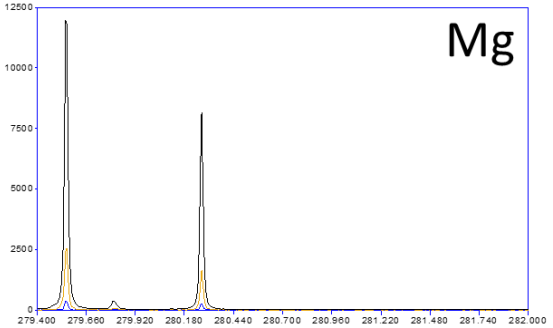
New Apparatus Data

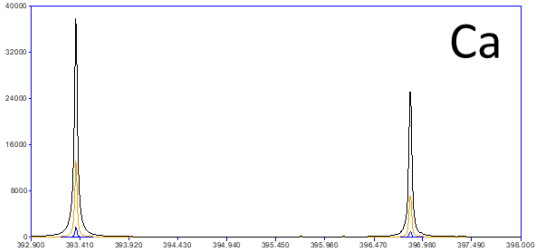


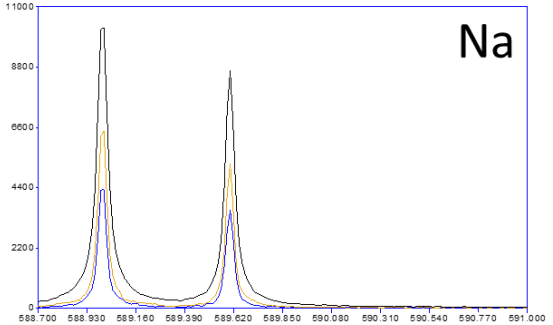
New Apparatus Data











University of Windsor

Detecting Infection in aCSF with a PLS-DA

Bacterial-negative aCSF specimens procured from a biochem supply vendor

Prepared Samples:

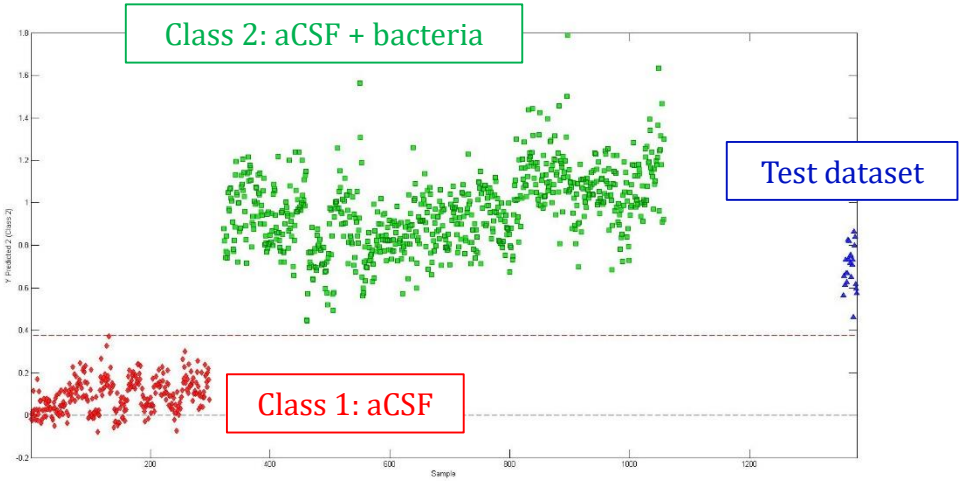
- 11 filters each of *S. aureus*, *E. coli*, *M. smegmatis*
- 13 filters of sterile aCSF
- 25 LIBS laser shots from each deposition

DETECTION IN aCSF (individual spectra)

100% sensitivity – 759 bacterial spectra
100% specificity – 299 aCSF spectra
11,000 cells per spectrum

DETECTION IN aCSF (spectra added)

33 of 33 filters positive, 100% sensitivity
13 of 13 filters negative, 100% specificity



This data was achieved by implementing outlier rejection in which two spectra (out of 25 spectra) from every filter with the lowest summed intensities were removed from the model. Therefore, **23** LIBS laser shots from each deposition were utilized in data analysis.

Diagnosing Infection in aCSF with PCA-ANN

Bacterial-negative aCSF specimens procured from a biochem vendor

Prepared Samples:

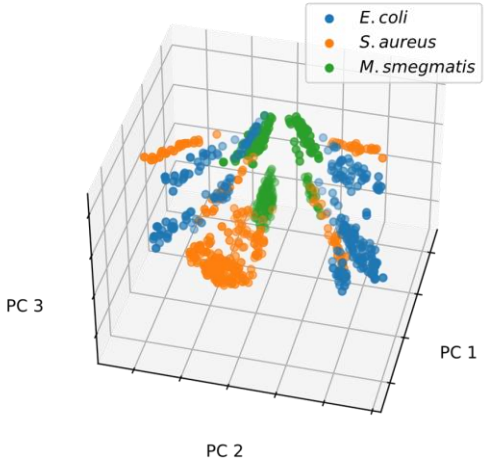
- 11 filters each of *S. aureus*, *E. coli*, *M. smegmatis*
- 13 filters of sterile aCSF
- 25 LIBS laser shots from each deposition

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 %



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

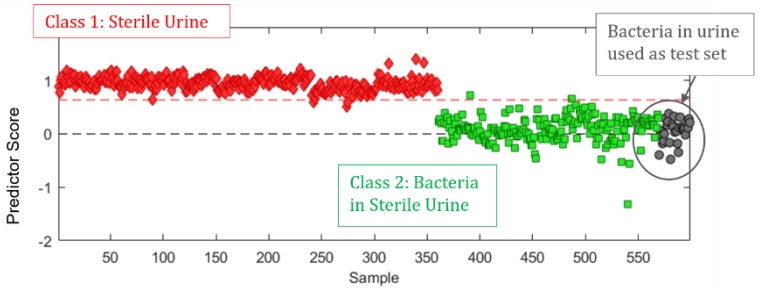
Conclusions

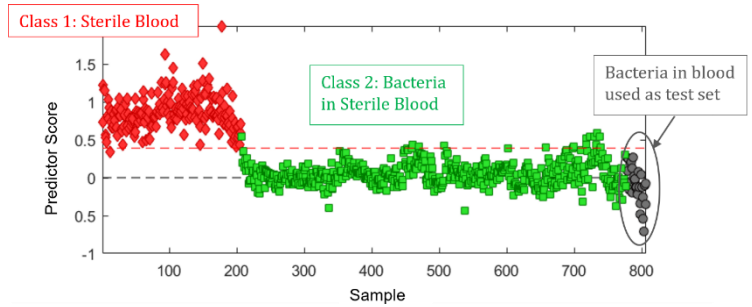
- Bacteria can be **detected** (PLSDA) and **diagnosed** (PCA-ANN) in blood, urine, and CSF.

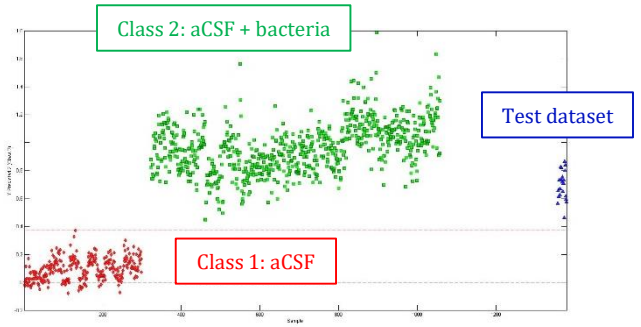
PCA-ANN With Full Spectrum Data (Urine)			
	S. aureus	E. coli	E. cloacae
Sensitivity	100 %	100 %	91.67 %
Specificity	100 %	95.83 %	100 %
Classification Error	0.00 %	2.09 %	4.17 %

PCA-ANN With Full Spectrum Data (Blood)				
	S. aureus	E. coli	E. cloacae	P. aeruginosa
Sensitivity	100 %	100 %	100 %	100 %
Specificity	100 %	100 %	100 %	100 %
Classification Error	0.00 %	0.00 %	0.00 %	0.00 %

PCA-ANN With Full Specrum Data (aCSF)				
	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 %

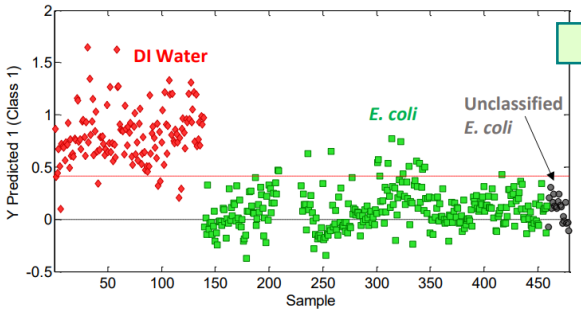




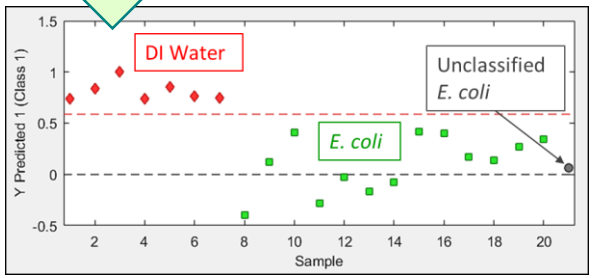


Conclusions

- Adding spectra improves discrimination in PLSDA
  - Detection of bacteria in water, blood, urine, and aCSF improved; **sensitivity = 100%**, **specificity = 100%**
- Rigorous cleaning of cone & usage of ultrapure water reduces background signal



Adding 30 single-shot spectra to create one measurement per filter improves classification in PLSDA



- Using PCA-ANN on full spectrum data provides the best results for discrimination between bacterial species (using 80:20 split)
- Average sensitivity = **94 %**
- Average specificity = **96 %**

PCA-ANN With Full Spectrum Data			
	E. coli	S. aureus	E. cloacae
Sensitivity	98.04 %	93.27 %	91.23 %
Specificity	97.71 %	97.22 %	96.12 %
Classification Error	2.13 %	4.28 %	6.33 %

Approximate increase from DFA ≈ 30 % (sensitivity), 16% (specificity)

Future Work

Improve external validation using PCA-ANN for bacterial species

- New 3D-printed centrifuge insert with integrated concentration cone help to improve signal-to-noise – revisit blood/urine.
- Work to further optimize the PCA-ANN algorithm (adding hidden layers?)
- Determine what is causing the "between-filter" variance in the measurements

Discrimination of lower concentrations of cells to find limit of identification (LOI)

- Engage microbiology students to investigate the behavior of cells in clinical specimens

Extend analysis to other bacterial strains

- *Mycobacterium tuberculosis* and *Streptococcus mitis*

If you want to learn more:

E.J. Blanchette. *Detection and Diagnosis of Bacterial Pathogens in Blood and Urine Using Laser-Induced Breakdown Spectroscopy*. Master's thesis, University of Windsor, 2022.

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* **76**, 2022, pp. 894-904. https://doi.org/10.1177/000370282210927

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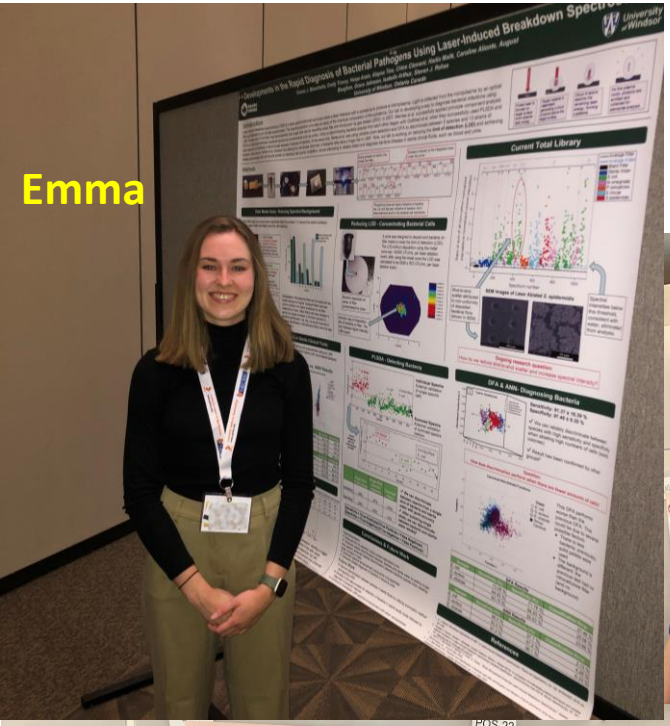
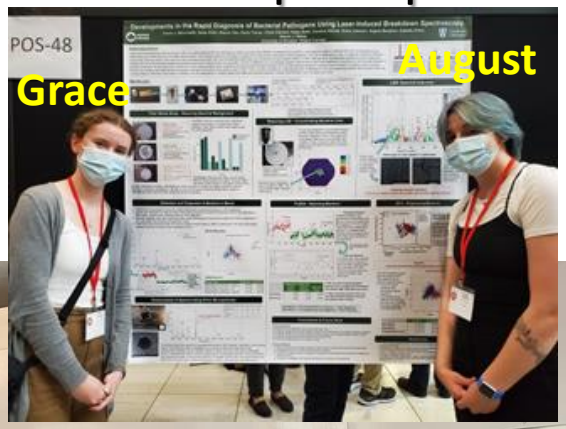
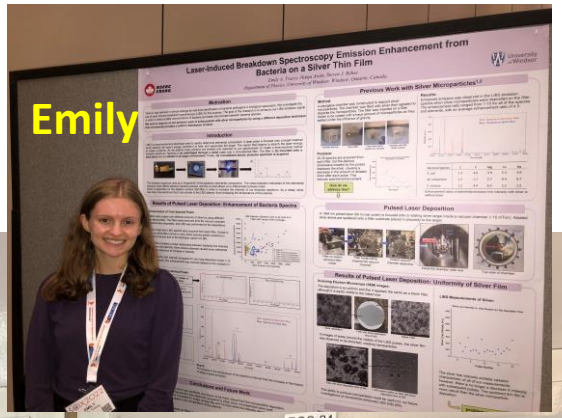
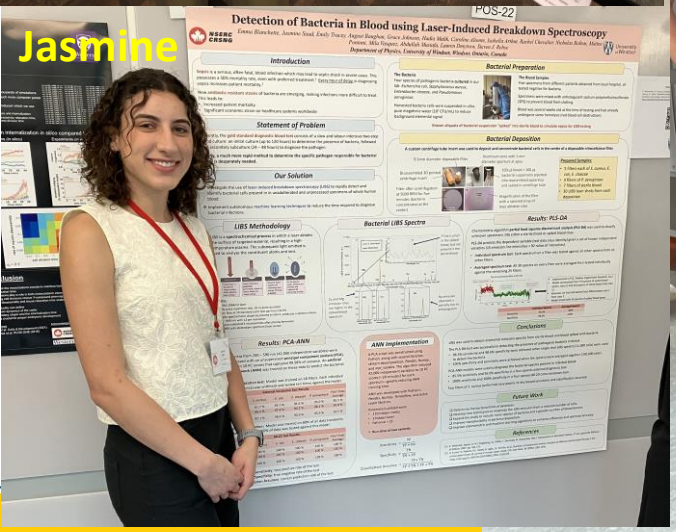
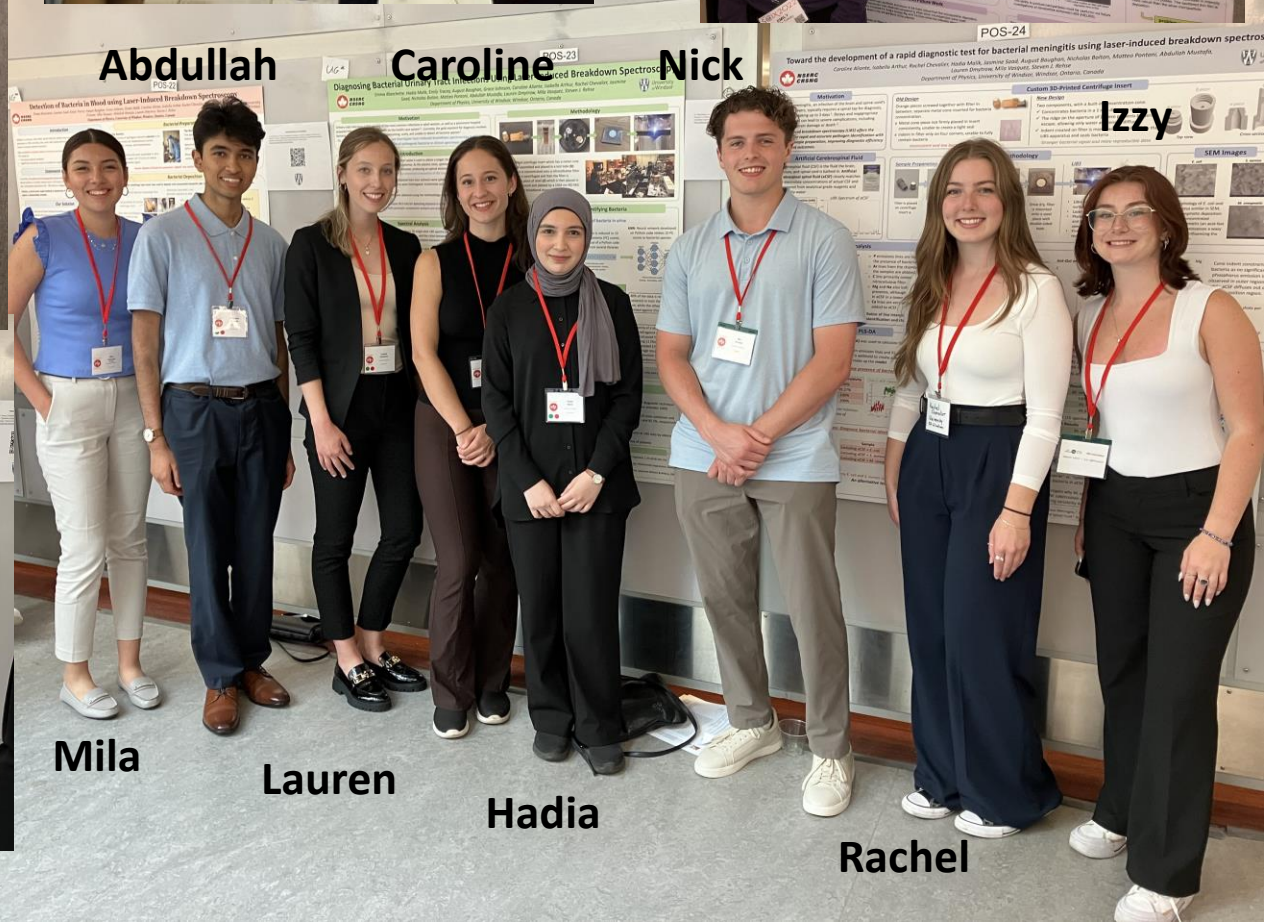
Acknowledgements for the people who did the work…











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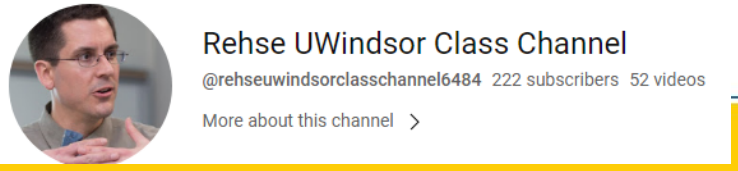

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