LASER-INDUCED BREAKDOWN SPECTROSCOPY FOR THE IDENTIFICATION OF PATHOGENS IN BLOOD AND URINE

Presented at CAP Congress 2023

Canadian Association of Physicists
SUPPORTING PHYSICS RESEARCH AND EDUCATION IN CANADA

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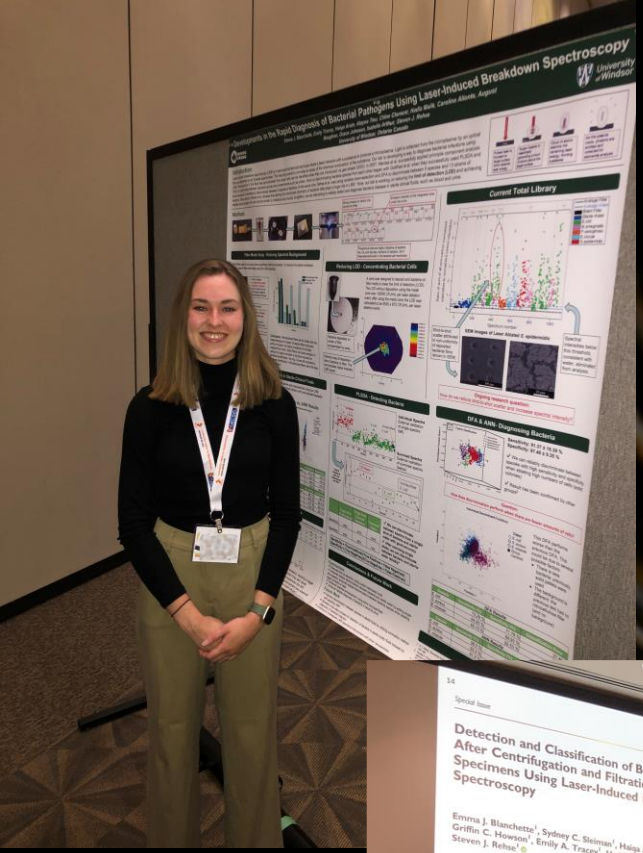

Emma at SciX 2022

This work defended by Master's student Emma Blanchette, December 2022.

Emma received a 48TH IEEE ICOPS "Best Student Paper" award, September 2021; placed 2nd in the DAMOP-C student oral presentation competition at CAP2022.

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.

Emma at CAP 2022

Funding and Acknowledgements

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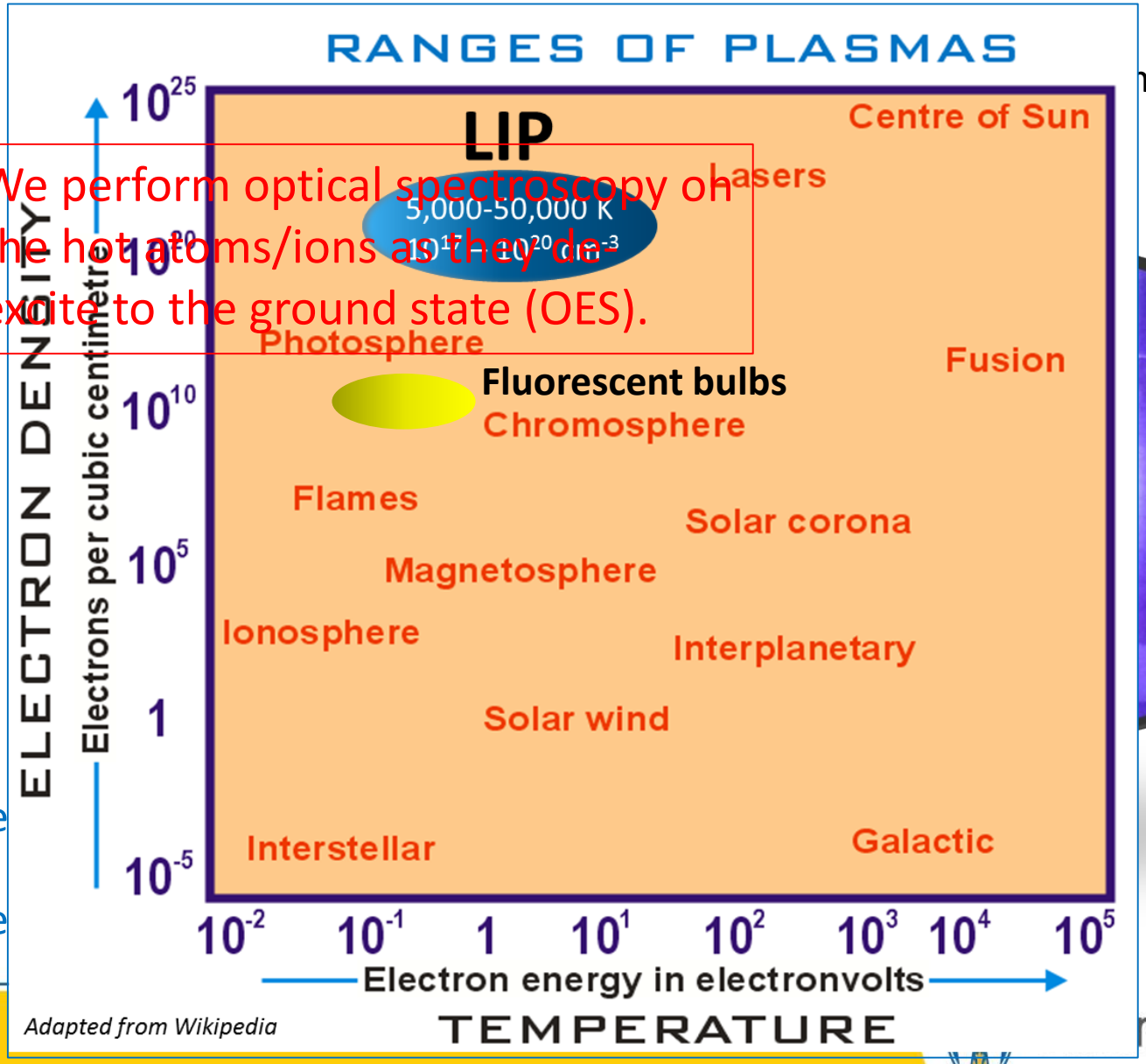
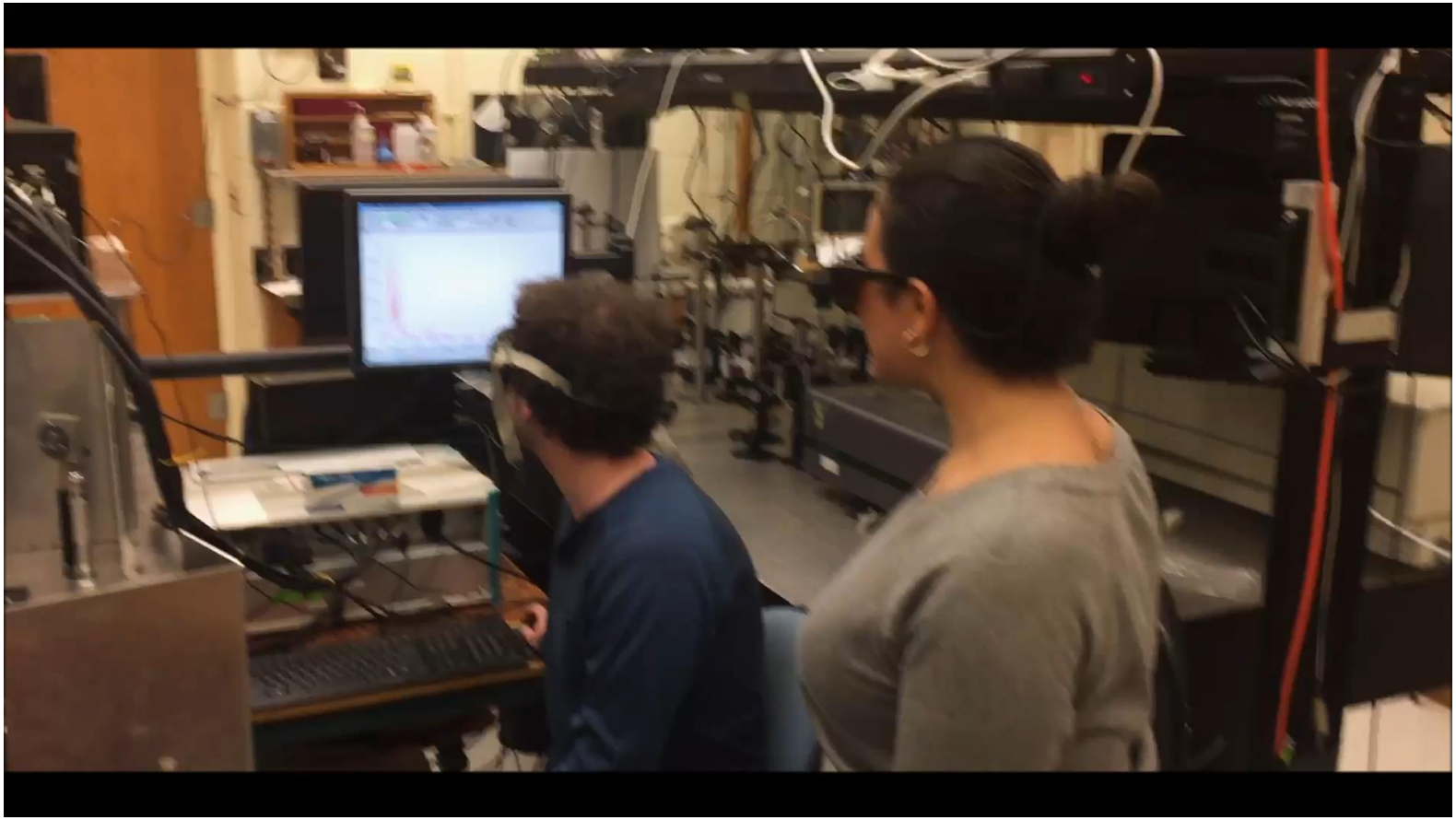
- A ***Natural Sciences and Engineering Research Council of Canada*** Discovery grant and a Research Tools and Instruments grant
- A ***Canada Foundation for Innovation*** Opportunity Fund grant
- An ***Ontario Research Fund*** Small Infrastructure Funds grant
- ***University of Windsor*** Outstanding Scholars program
- ***University of Windsor*** Faculty of Science

What is “Laser-Induced Breakdown Spectroscopy?”

We perform optical spectroscopy on the hot atoms/ions as they de-excite to the ground state (OES).

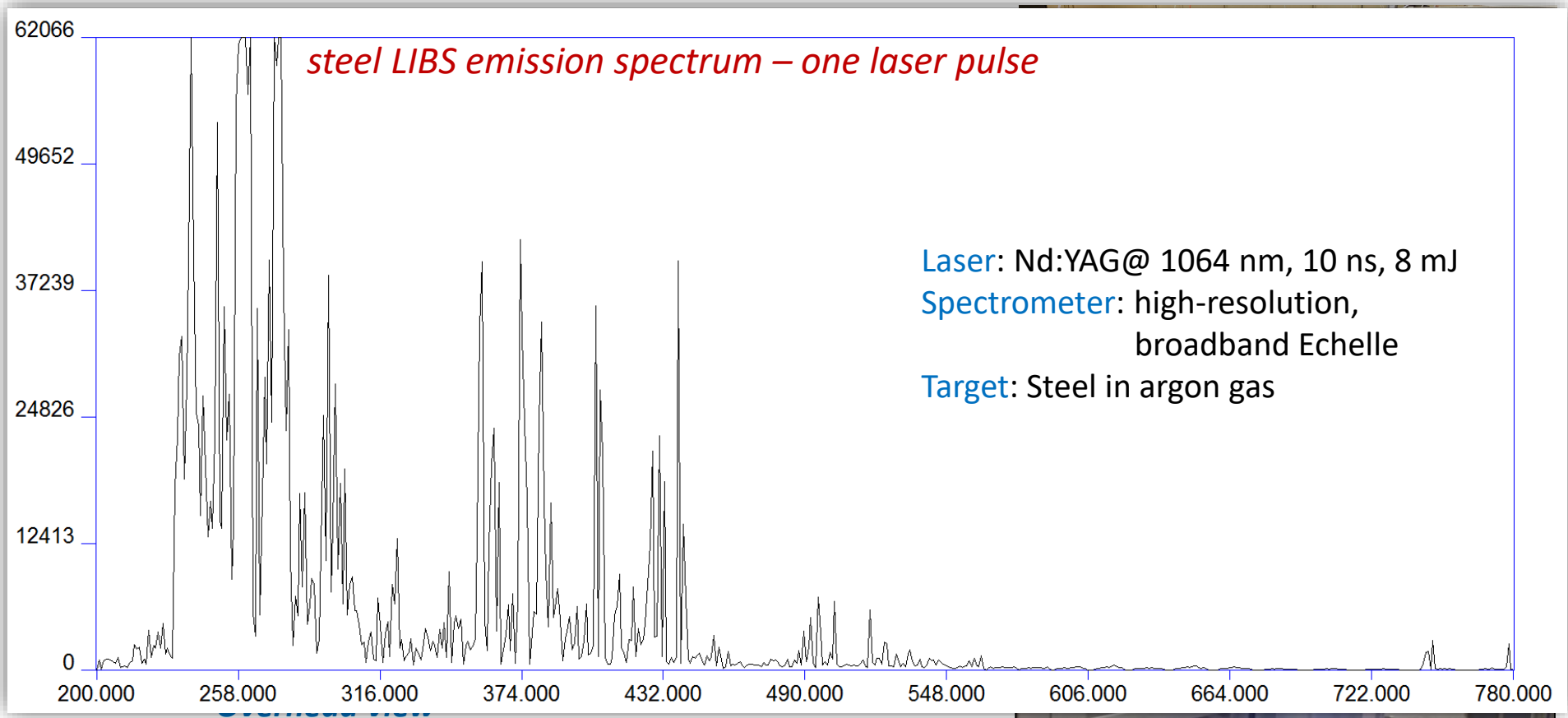


- Can be
- Can be



Experimental Apparatus

Optical Tables

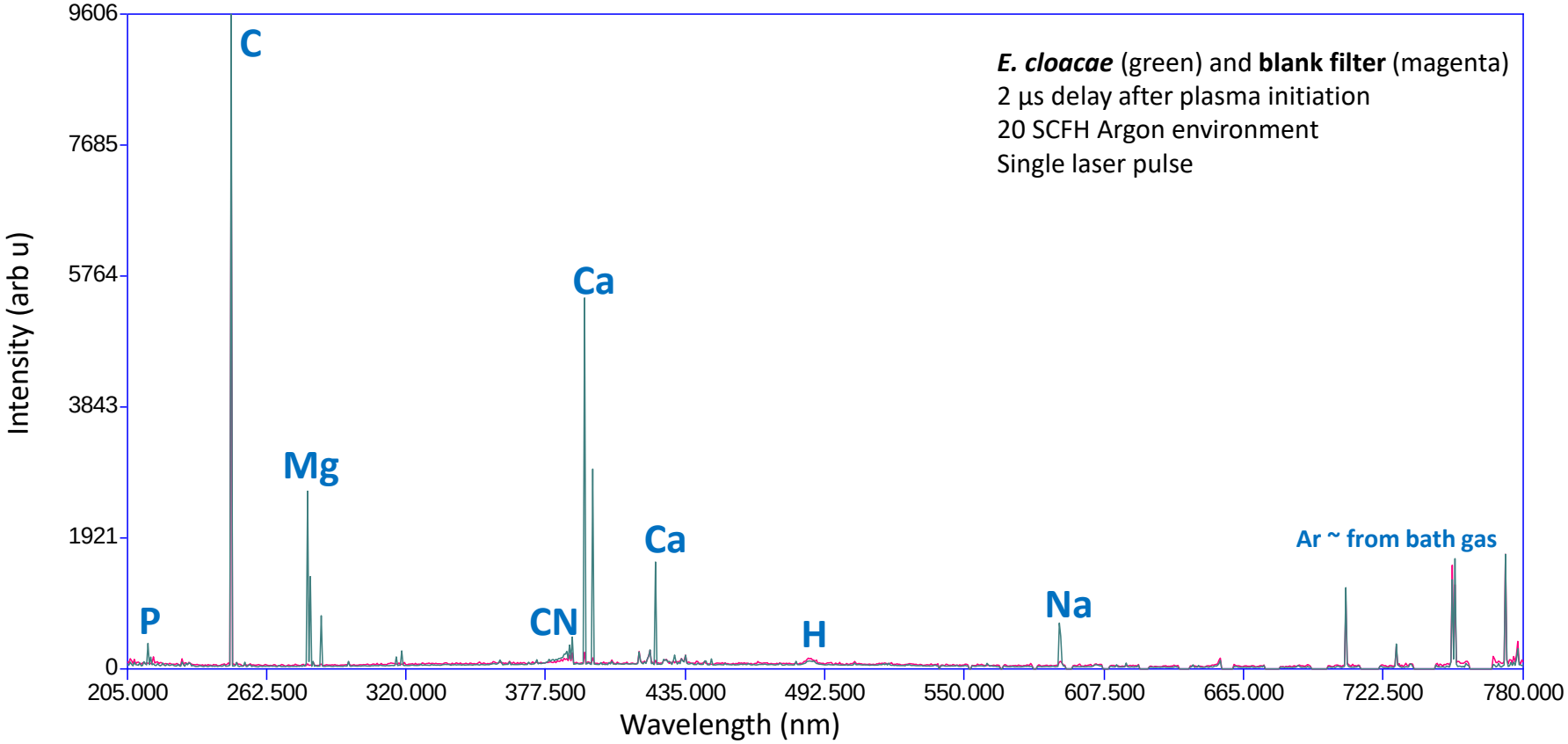


Laser: Nd:YAG@ 1064 nm, 10 ns, 8 mJ
Spectrometer: high-resolution, broadband Echelle
Target: Steel in argon gas

Steel plasma in argon

Spectrum From Bacteria on Nitrocellulose Filter

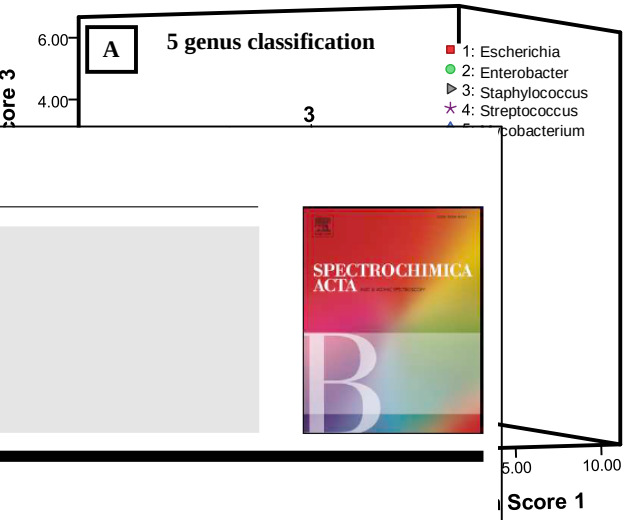
An elemental assay of the bacterial cell composition!



Suggests a real-time method for pathogenic bacterial diagnosis.

Already Shown…

- ✓ We can identify a bacterial species, certainly its genus, with high sensitivity and specificity (confirmed by others).



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Invited Review

A review of the use of laser-induced breakdown spectroscopy for bacterial classification, quantification, and identification

Steven J. Rehse*

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Positive	54.05%	0.51%
Negative	99.49%	45.95%

Positive	86.49%	5.85%
Negative	94.15%	13.51%

STREPTOCOCCUS	True	False
Positive	95.59%	1.02%
Negative	98.98%	4.41%

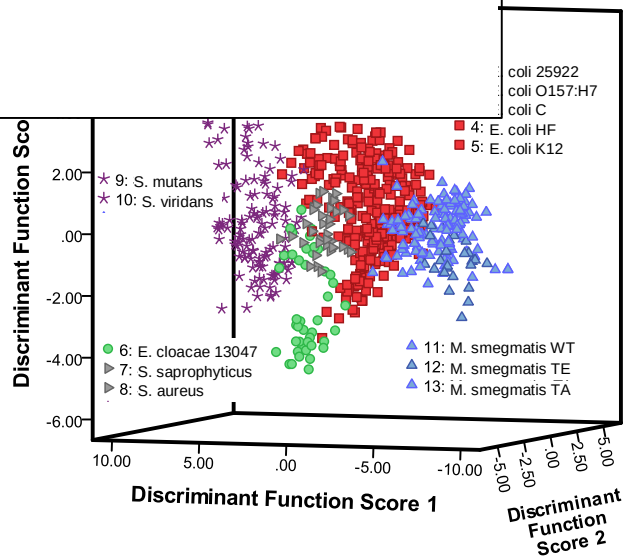
STREPTOCOCCUS	True	False
Positive	99.26%	13.32%
Negative	88.68%	0.74%

MYCOBACTERIUM	True	False
Positive	88.31%	1.06%
Negative	98.94%	11.69%

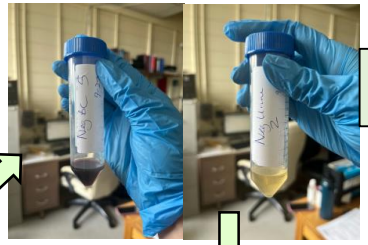
MYCOBACTERIUM	True	False
Positive	96.10%	4.08%
Negative	95.92%	3.90%

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

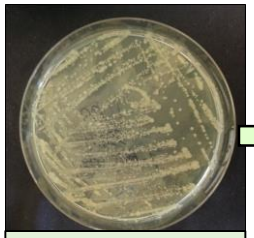
PLSDA: *Sensitivity*: 93.13 ± 10.25 % *Specificity*: 90.60 ± 21.33 %



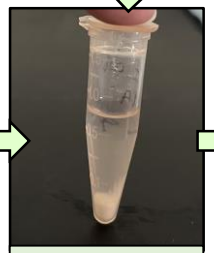
Methodology – Bacterial Growth & Sample Prep



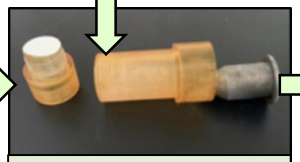
Bacterial negative specimens from hospital



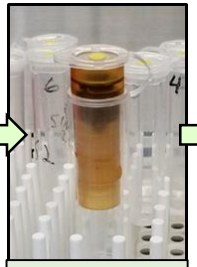
Bacteria grown on agar plate



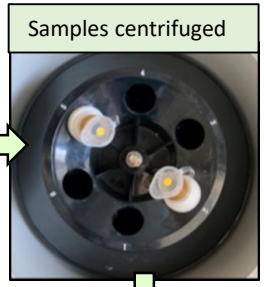
Harvested bacteria stored in $M\Omega H_2O$



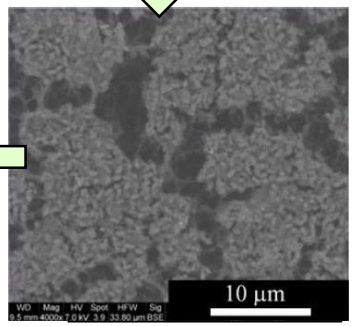
Centrifuge insert assembled with filter & cone

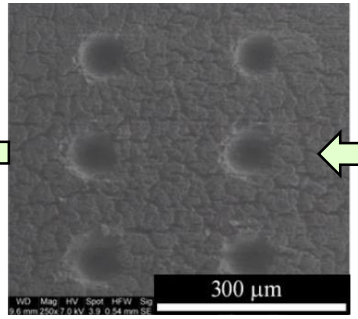


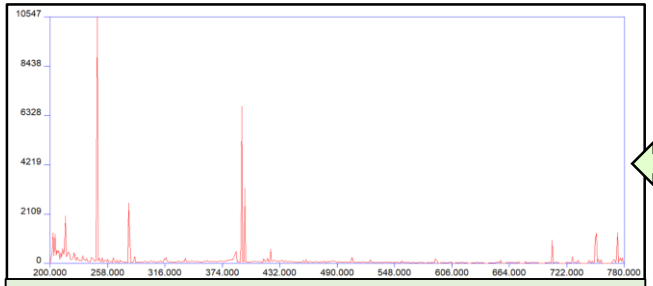
Assembled piece placed in centrifuge tube



Samples centrifuged

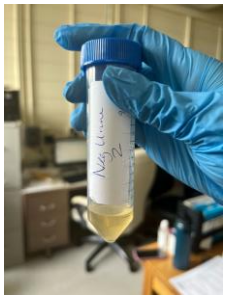




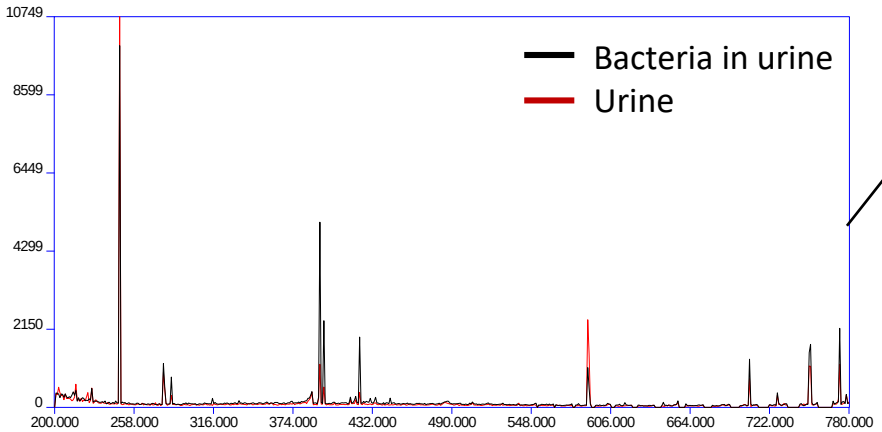


Spectrum of bacterial sample

Diagnosing Infection (Detecting Bacteria) in Urine



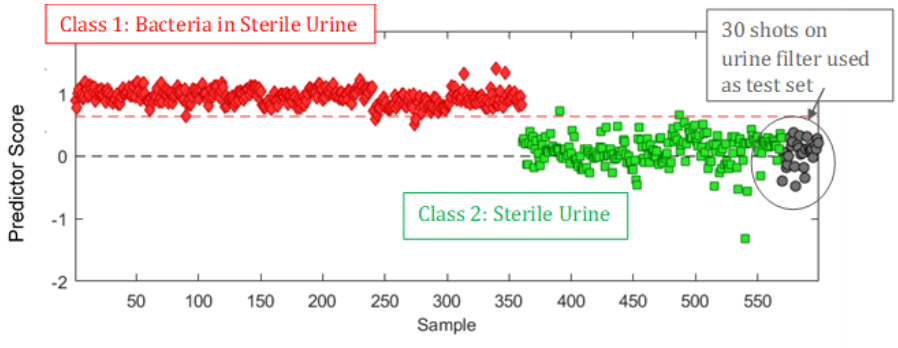
Bacterial-negative specimens obtained from Windsor Regional Hospital



DETECTION IN URINE

98.9% sensitivity

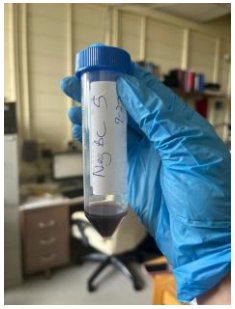
100% specificity



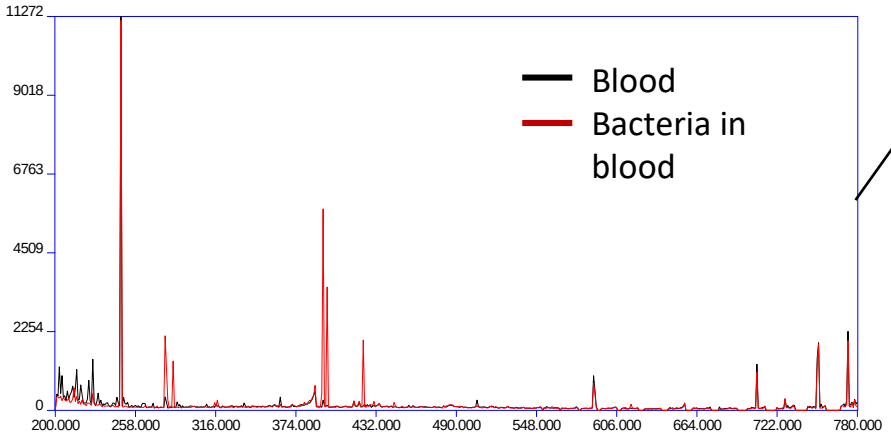
- 360 spectra of bacteria in urine
  - 3 species, four filters of each
  - *S. aureus, E. coli, E. cloacae*
- 240 spectra of sterile urine
  - 8 filters from multiple patients
- Each filter sequentially removed from the model for “external testing”

University of Windsor

Diagnosing Infection (Detecting Bacteria) in Blood



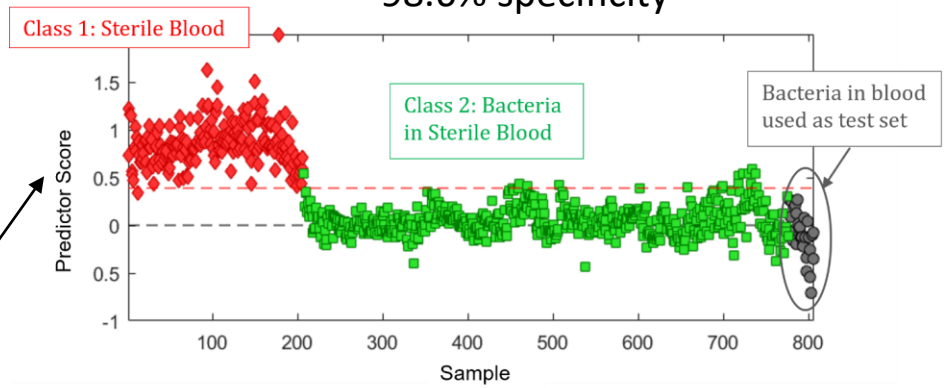
Bacterial-negative specimens obtained from Windsor Regional Hospital



DETECTION IN BLOOD

96.3% sensitivity

98.6% specificity



- 600 spectra of bacteria in blood
  - 4 species, five filters of each
  - *S. aureus, E. coli, E. cloacae, P. aeruginosa*
- 206 spectra of sterile urine
  - 7 filters from multiple patients
- Each filter sequentially removed from the model for "external testing"

Diagnosing Species in Water with Machine Learning

"Ratio Model" consists of 15 emission line intensities and 92 simple ratios. 107 independent variables.

DFA on Ratio Model

ANN on Ratio Model

PCA-ANN on Ratio Model

PCA-ANN on Full Spectrum Data*

- 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.
- Models are trained on 80% of single shot data, 20% reserved for testing. (~15 seconds).

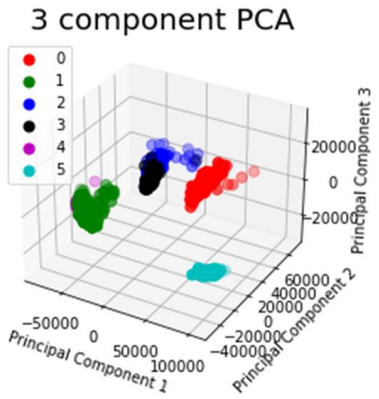
PCA-ANN With Full Spectrum Data			
	E. coli	S. aureus	E. cloacae
Sensitivity	98.04 %	93.27 %	91.23 %
Specificity	97.71 %	97.22 %	96.12 %
Classification Error	2.13 %	4.28 %	6.33 %

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

Diagnosing Species in Water with Machine Learning

- PCA-ANN custom written in Python
- 42,000 independent variables ⇒ 10 principal components (new independent variables)
- Currently only one hidden layer
- ANN algorithm implemented to optimize the patience and # of hidden nodes
- Models are trained on 80% of single shot data, 20% reserved for testing.

- After PCA, spectra were randomized to demonstrate the ANN was not fitting noise (random classification of cases)



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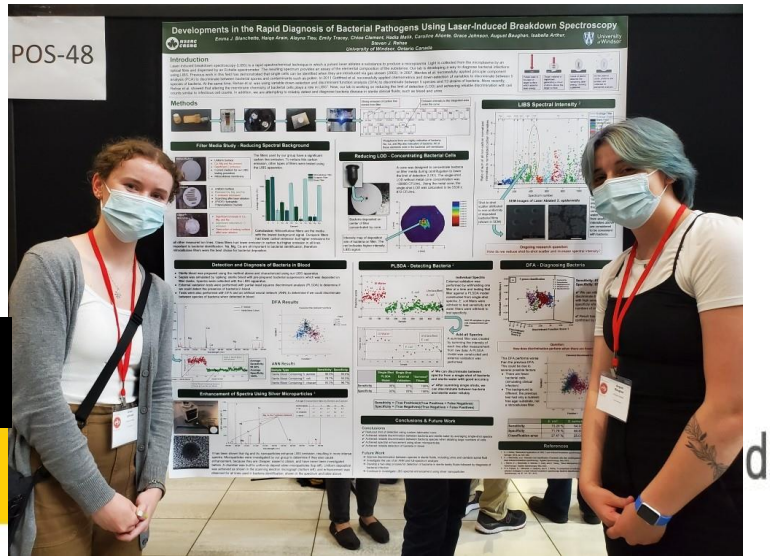

Grace and August at CAP 2022

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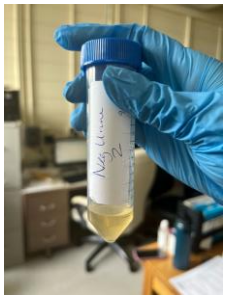
Diagnosing Infection in Urine with an ANN



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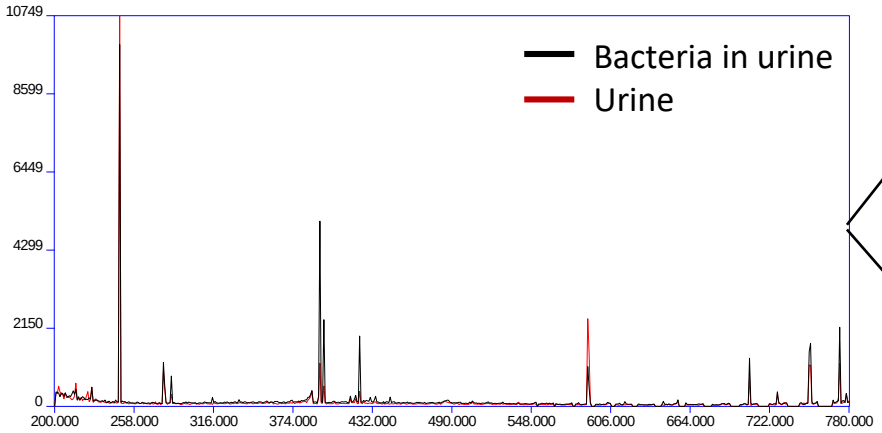

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- 240 spectra of sterile urine
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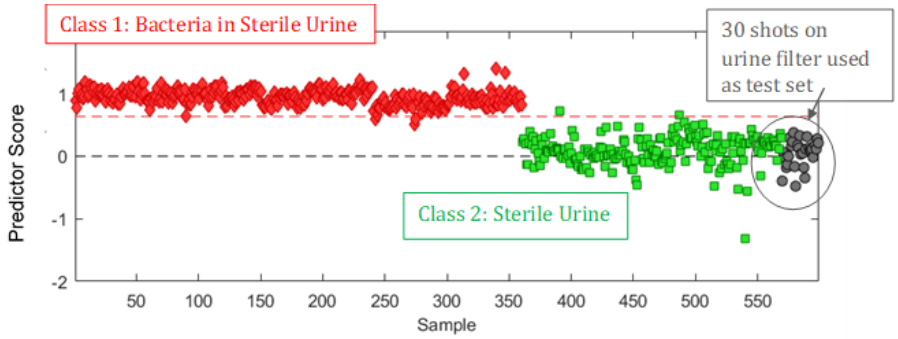
DETECTION IN URINE

98.9% sensitivity

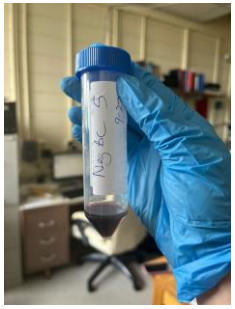
100% specificity



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

Diagnosing Infection in Blood with an ANN



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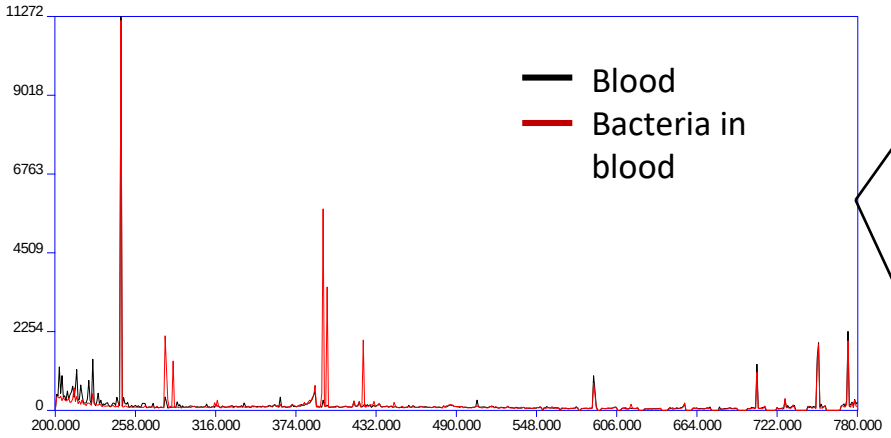

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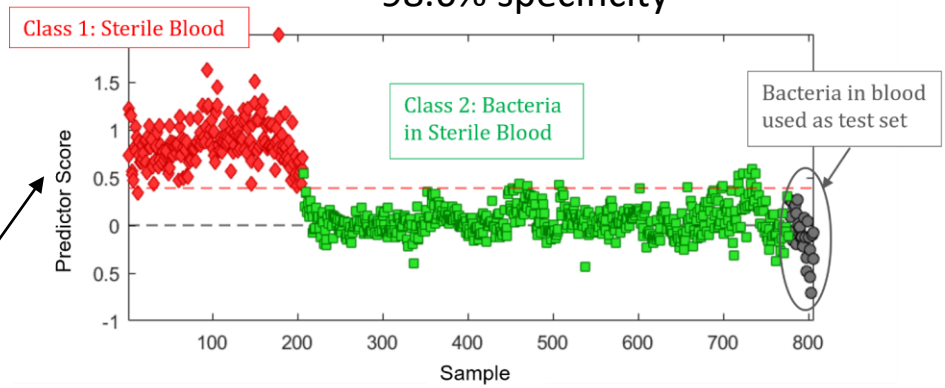
  - 7 filters

DETECTION IN BLOOD

96.3% sensitivity

98.6% specificity



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

External Validation of PCA-ANN in Blood and Urine

- External validation diagnosis performed in both urine (3 species) and blood (4 species)
- Sensitivities drop from ~100% to ~80%

average sensitivity of 77.5%

Urine	***E. coli***	***S. aureus***	***E. cloacae***	
Average Sensitivity	75.83 %	90.00 %	66.67 %	
Blood	***E. coli***	***S. aureus***	***E. cloacae***	***P. aeruginosa***
Average Sensitivity	80.67 %	65.33 %	92.67 %	92.50 %

average sensitivity of 82.3%

Urine Example

E. coli	Predicted			
Sample #	*S. aureus*	*E. coli*	*E. cloacae*	Sensitivity
1	0	28	2	93.333333
2	2	8	20	26.666667
3	0	25	5	83.333333
4	0	30	0	100
Sum	2	91	27	75.833333

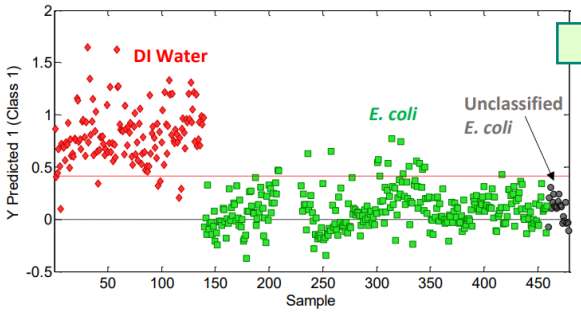
Blood Example

S. aureus	Predicted				
Sample #	*S. aureus*	*E. coli*	*E. cloacae*	*P. aeruginosa*	Sensitivity
1	30	0	0	0	1
2	1	0	29	0	0.0333333
3	30	0	0	0	1
4	30	0	0	0	1
5	7	23	0	29	0.2333333
Sum	98	23	29	0	0.6533333

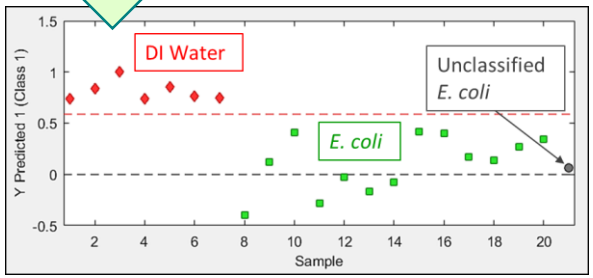
✓ Improvements need to be made on external validation

Conclusions

- Adding spectra improves discrimination in PLSDA
  - Detection of bacteria in water 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 %

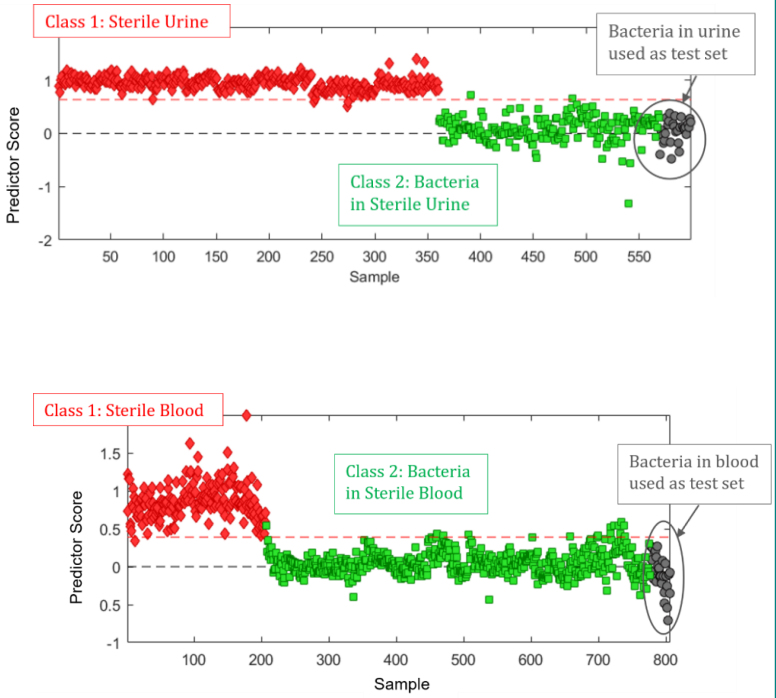
Approximate increase from DFA ≈ 30 % (sensitivity), 16% (specificity)

Conclusions

- Bacteria can be **detected** (PLSDA) and **diagnosed** (PCA-ANN) in blood and urine
- Future work:
  - Determine LOD for bacteria in blood and urine
  - Study how bacteria behaves in blood to ensure we are reproducing clinical conditions
  - Apply new deposition method to blood & urine

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 %



Future Work

Improve external validation using PCA-ANN for bacterial species

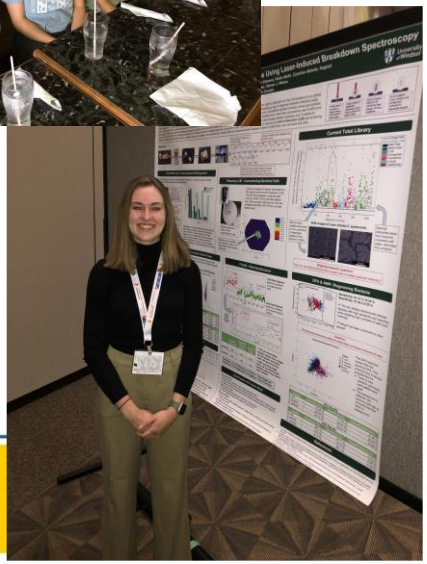
- New 3D-printed centrifuge insert with integrated concentration cone will help to improve signal-to-noise
- Work to further optimize the PCA-ANN algorithm (adding hidden layers?)

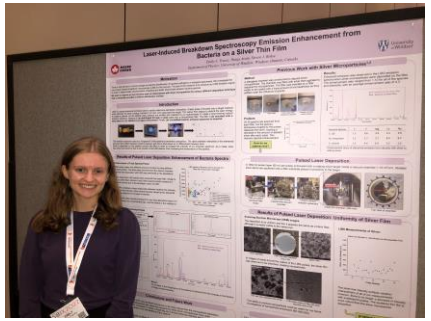
Discrimination of lower concentrations of cells to find limit of identification (LOI)

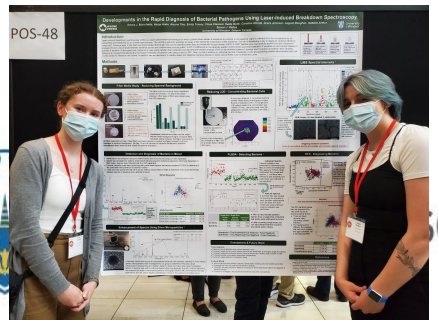
- Engage microbiology students to investigate the behavior of cells in clinical specimens

Acknowledgements for the people who did the work…

2022-2023



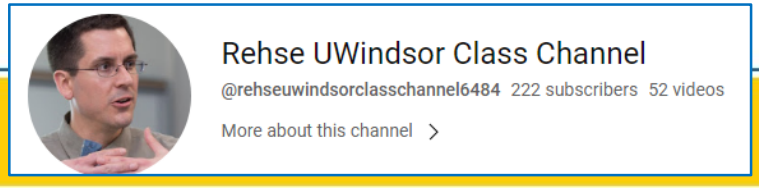




2021-2022

Thank you!

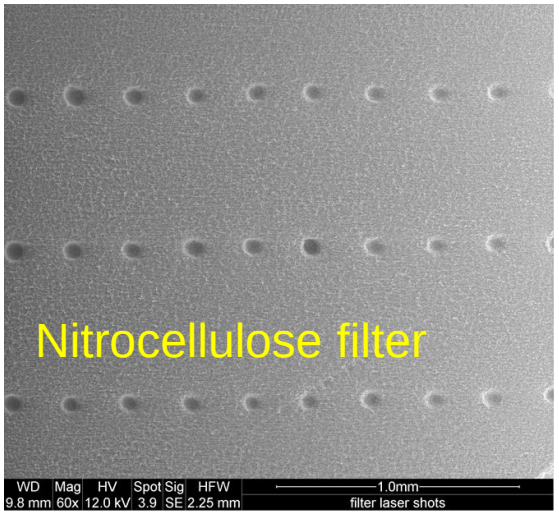
YouTube

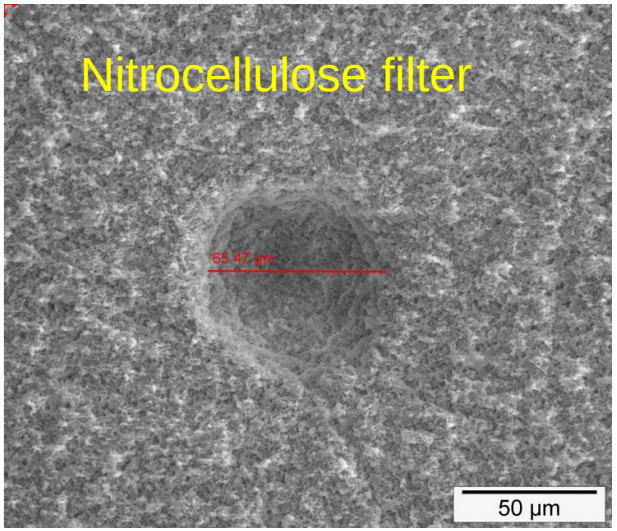


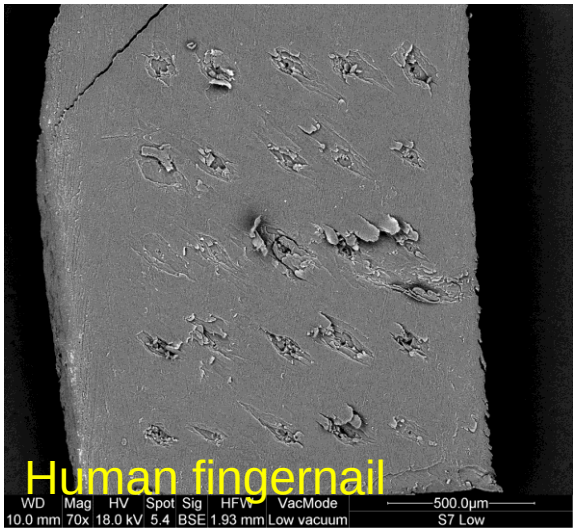
Webpage: www.uwindsor.ca/people/rehse/

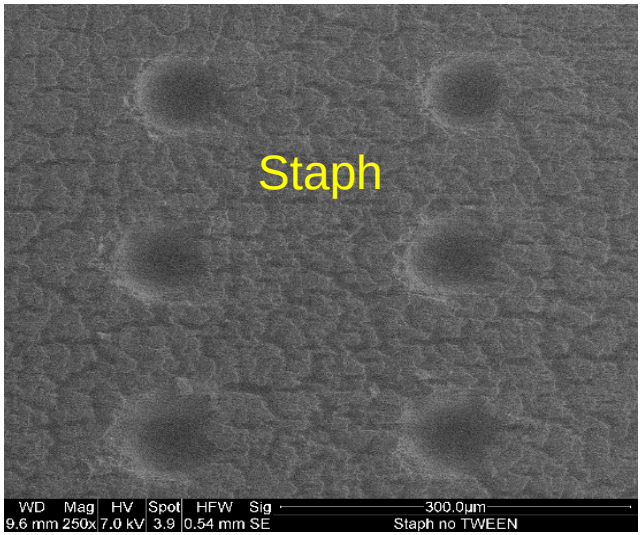
University of Windsor

2) removal of samples mass (ablation)



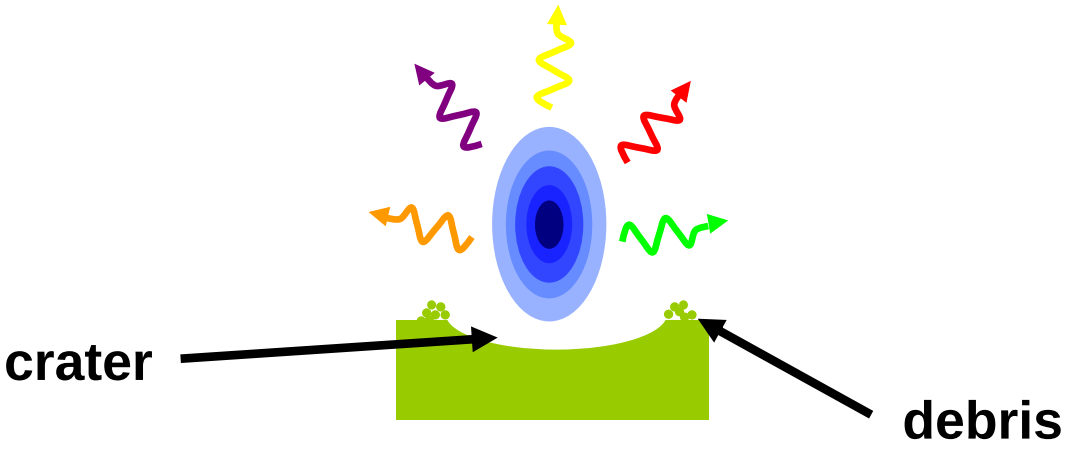




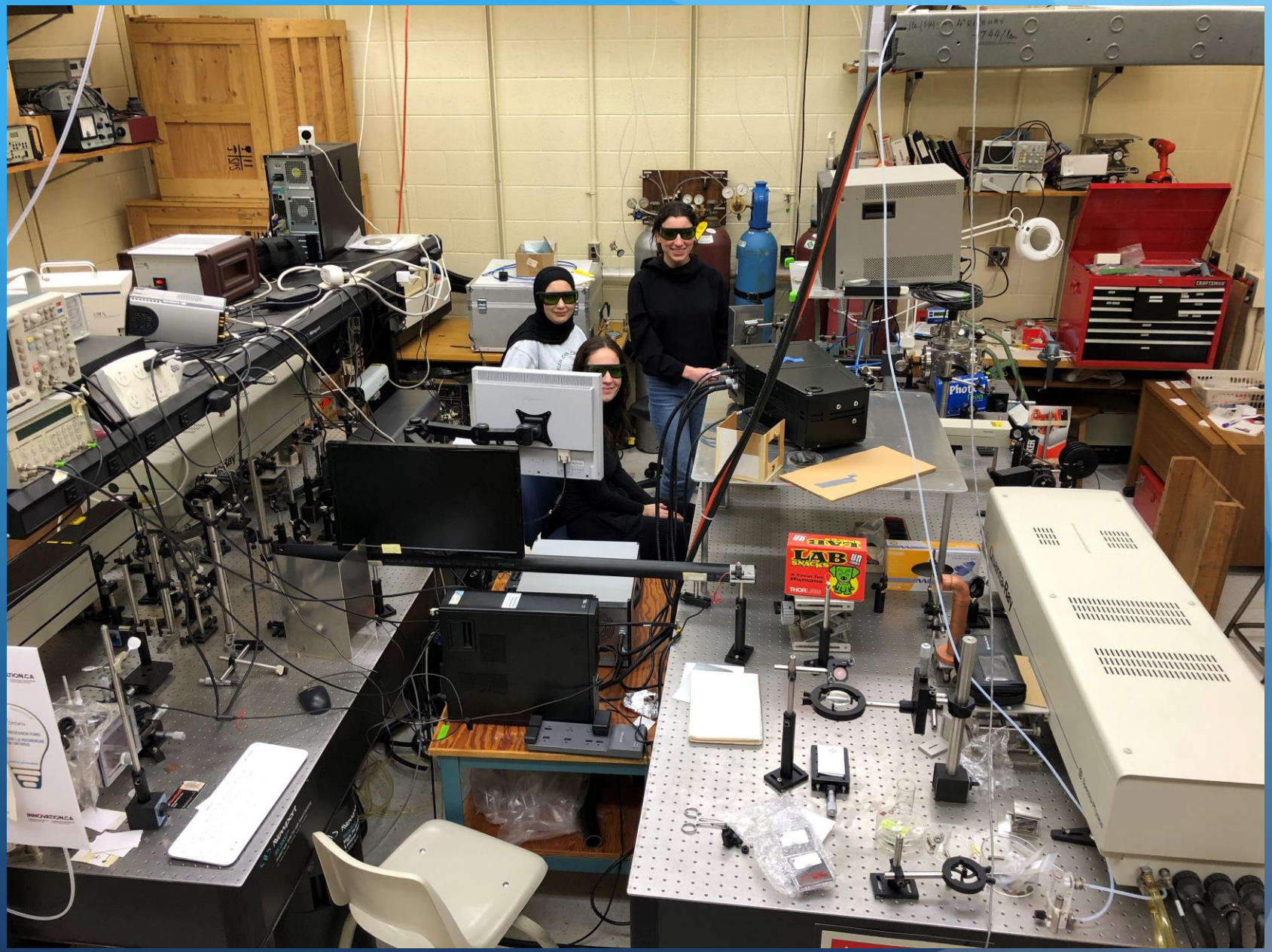


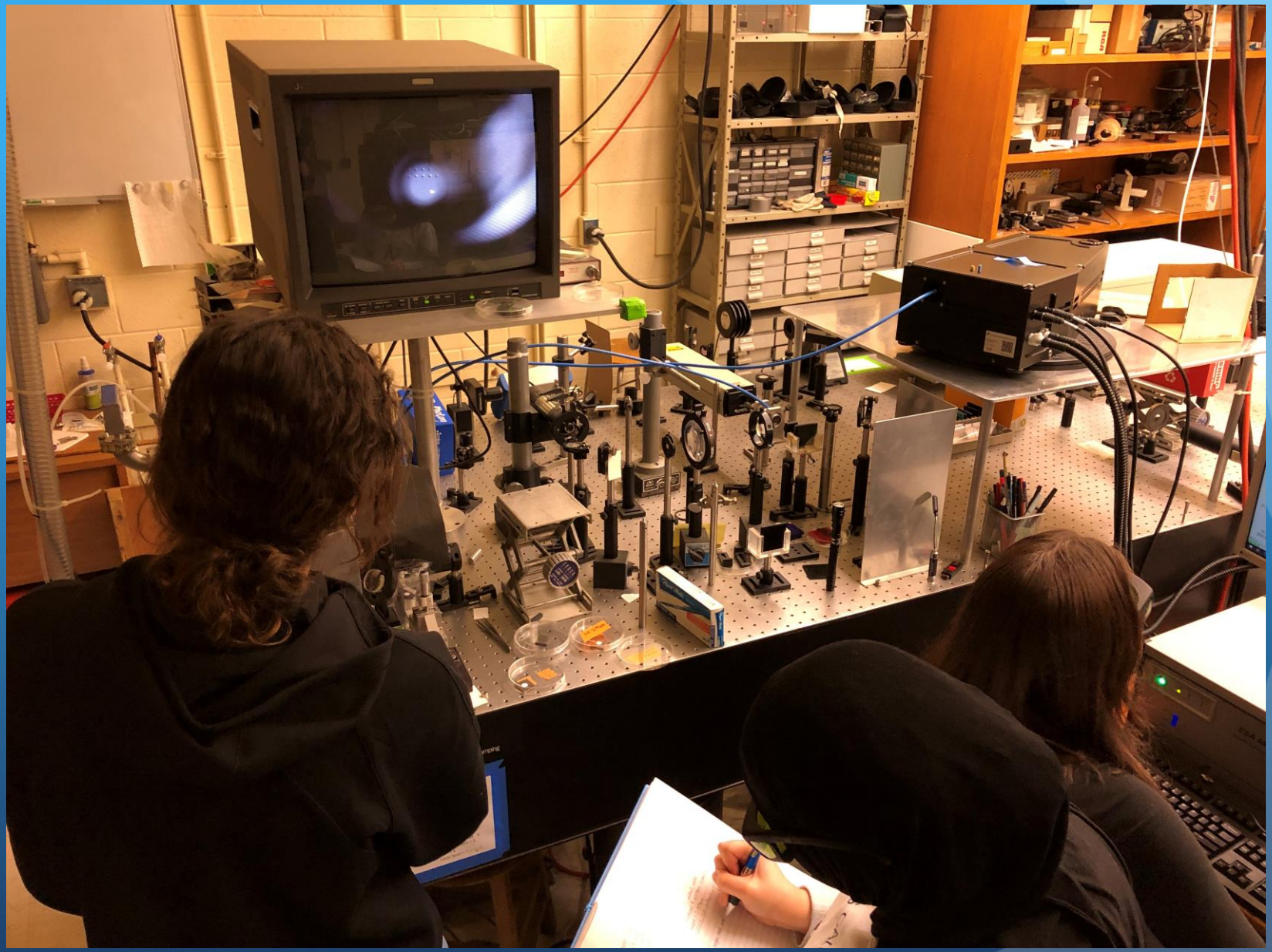
4) expansion and element specific emission (atomic or ionic)

spontaneous emission as atoms/ions decay to ground state

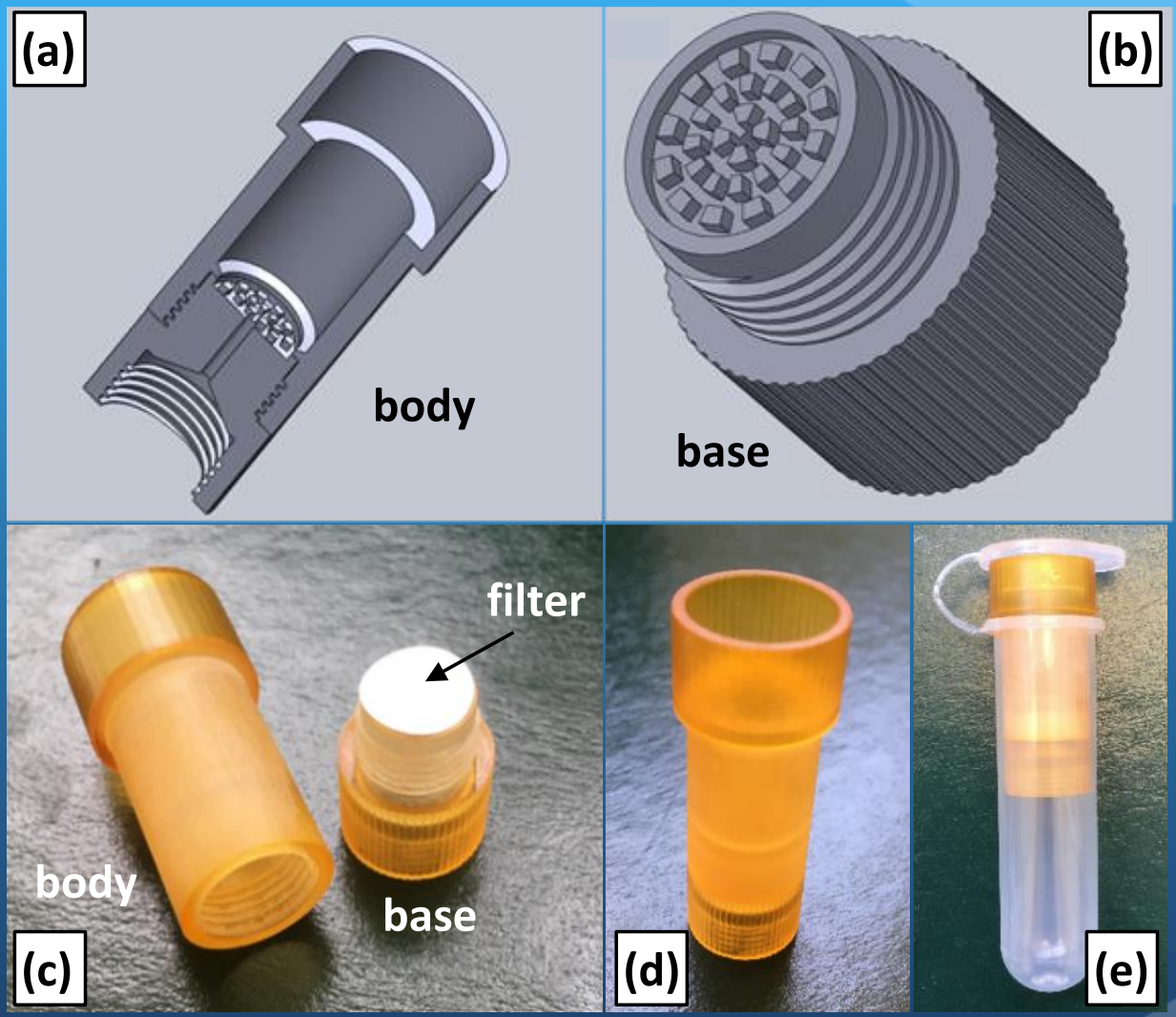


- The dynamic evolution of the plasma plume is then characterized by a fast expansion and subsequent cooling.
- Approximately 1 microsecond after the ablation pulse, spectroscopically narrow atomic/ionic emissions may be identified in the spectrum.


LAB
SNACKS



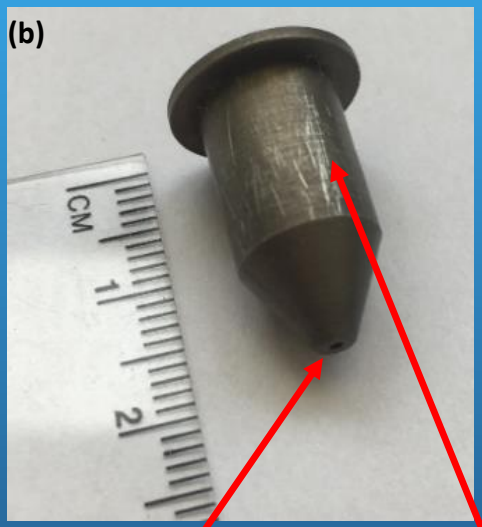
The Centrifuge Insert



Concentrating Bacteria With a Cone

19 mm long Al cone

Centrifuge tube cap presses cone into filter

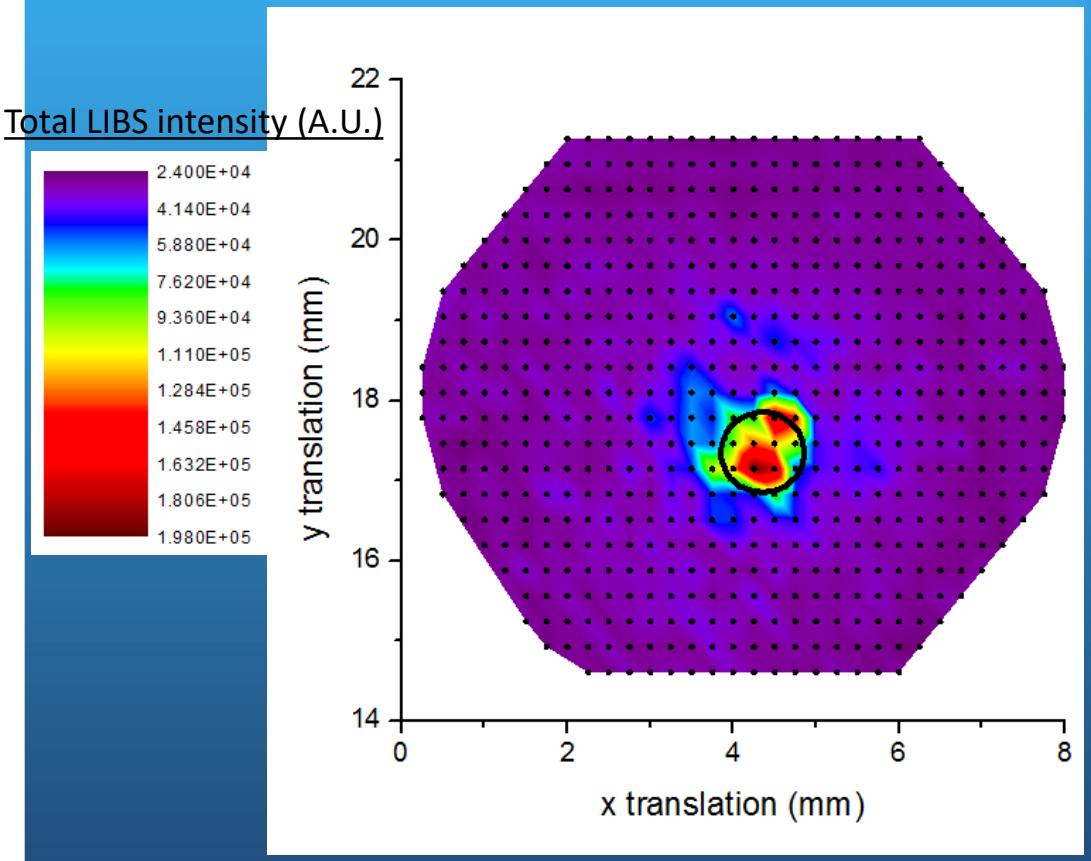


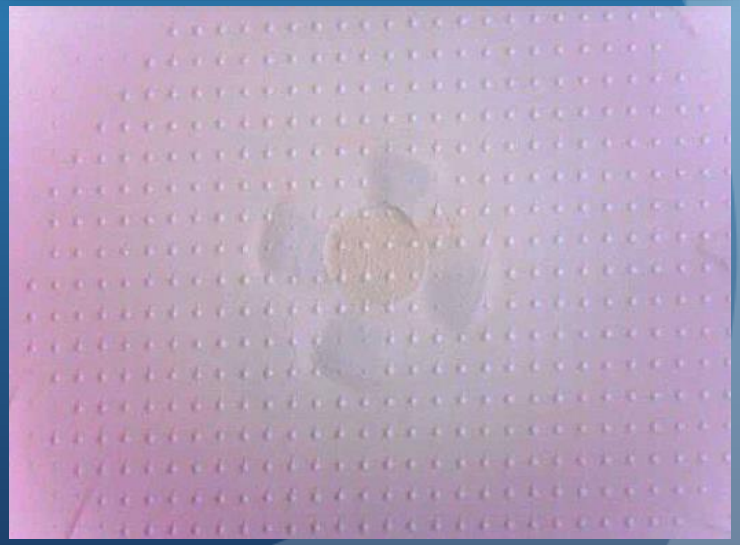
1 mm hole at apex

Holds 1 mL of fluid

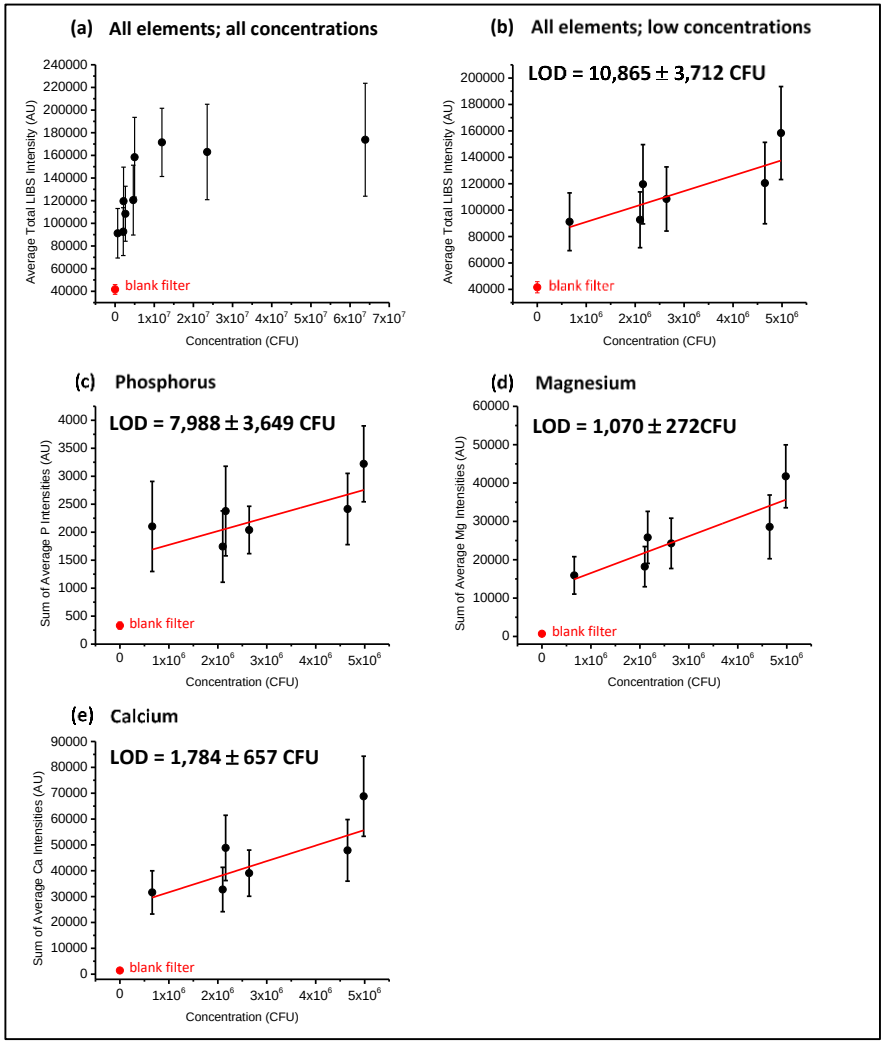
Cone vertex press fit into filter

Shooting Bacteria Concentrated With Cone





Limits of Detection



A calibration curves constructed from forty spectra obtained from each of nine different concentrations.

LIBS bacterial limit of detection of 10,865 ± 3,712 CFU per laser ablation event for bacteria deposited on filters using the metal cone.

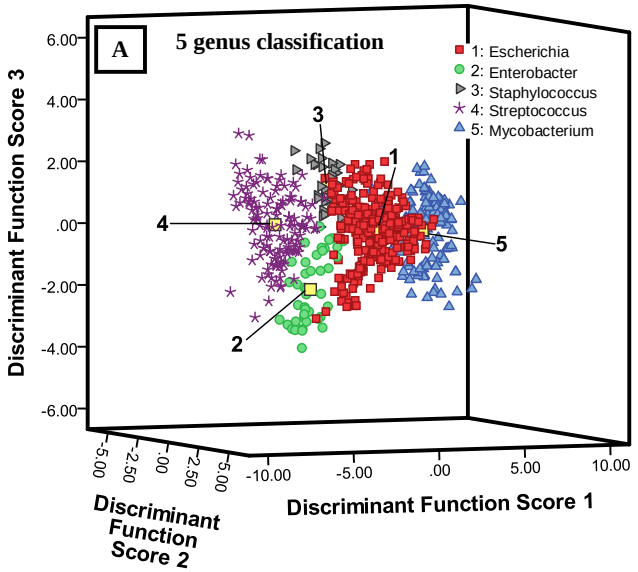
LOD's calculated using only certain elements observed in the LIBS spectra and present in very low concentrations in the filter were even lower:

1,070 ± 272CFU for magnesium
1,784 ± 657 CFU for calcium.

LOD on filter better, but number of cells required in fluid specimen is **WAY** lower!

When performed with no background

- ✓ We can identify a bacterial species, certainly its genus, with high sensitivity and specificity (confirmed by others).
- ✓ We can differentiate strains of *E. coli* (demonstrated by others in MRSA).
- ✓ Multiple multivariate techniques effective at discriminating spectra.



PLSDA

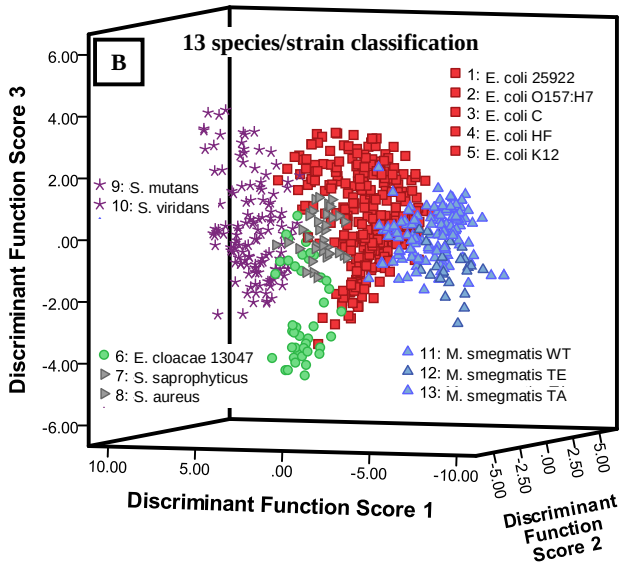
E. COLI	True	False
Positive	95.65%	9.17%
Negative	90.83%	4.35%
STAPHYLOCOCCUS	True	False
Positive	54.05%	0.51%
Negative	99.49%	45.95%
STREPTOCOCCUS	True	False
Positive	95.59%	1.02%
Negative	98.98%	4.41%
MYCOBACTERIUM	True	False
Positive	88.31%	1.06%
Negative	98.94%	11.69%

DFA

E. COLI	True	False
Positive	89.63%	15.95%
Negative	84.05%	10.37%
STAPHYLOCOCCUS	True	False
Positive	86.49%	5.85%
Negative	94.15%	13.51%
STREPTOCOCCUS	True	False
Positive	99.26%	13.32%
Negative	88.68%	0.74%
MYCOBACTERIUM	True	False
Positive	96.10%	4.08%
Negative	95.92%	3.90%

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

PLSDA: *Sensitivity*: 93.13 ± 10.25 % *Specificity*: 90.60 ± 21.33 %



Results: We have already demonstrated…

- LIBS spectral fingerprint is a *sensitive* and *specific* (high rates of true positives, low rates of false positives) test to identify an unknown bacterial specimen or to differentiate between possible identifications
- This spectral fingerprint is *robust* and *reliable*, and exists through time (multiple tests spanning years on same strains of bacteria)
- In addition…

9 publications in Applied Physics Letters, Journal of Applied Physics, Applied Optics, Applied Spectroscopy, Spectrochimica Acta B, and others – confirmed by multiple other groups

Results: We have already demonstrated…

LIBS spectral fingerprint is:

- growth-medium independent
- independent of state of growth (how “old” the bacteria are)
- independent of whether the bacteria are live or dead (or inactivated by UV light)
- obtainable even when other types of bacteria or contaminants are present (mixed samples)
- obtainable from urine specimens
- capable of strain discrimination
- obtainable from about 500 bacteria

9 publications in Applied Physics Letters, Journal of Applied Physics, Applied Optics, Applied Spectroscopy, Spectrochimica Acta B, and others – confirmed by multiple other groups

(Poster 344) **Bacterial Mounting and Concentration Techniques to Translate Laser-Induced Breakdown Spectroscopy into a Clinical Setting**; Alexandra Paulick