

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

Presented at ICOPS2023



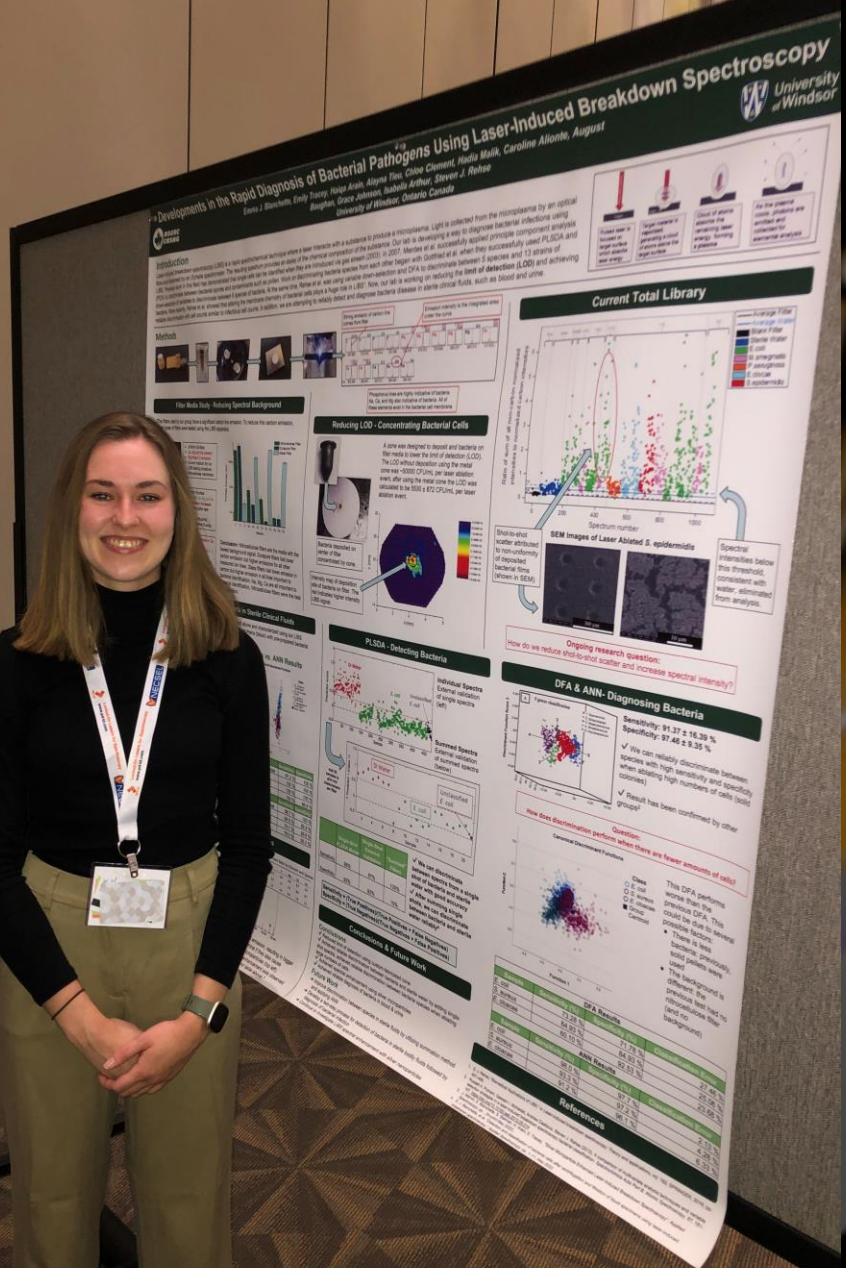
Steven J. Rehse

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University of Windsor

Windsor, Ontario, Canada



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

Emma received a 48TH IEEE ICOPS "Best Student Paper" award, September 2021.

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.

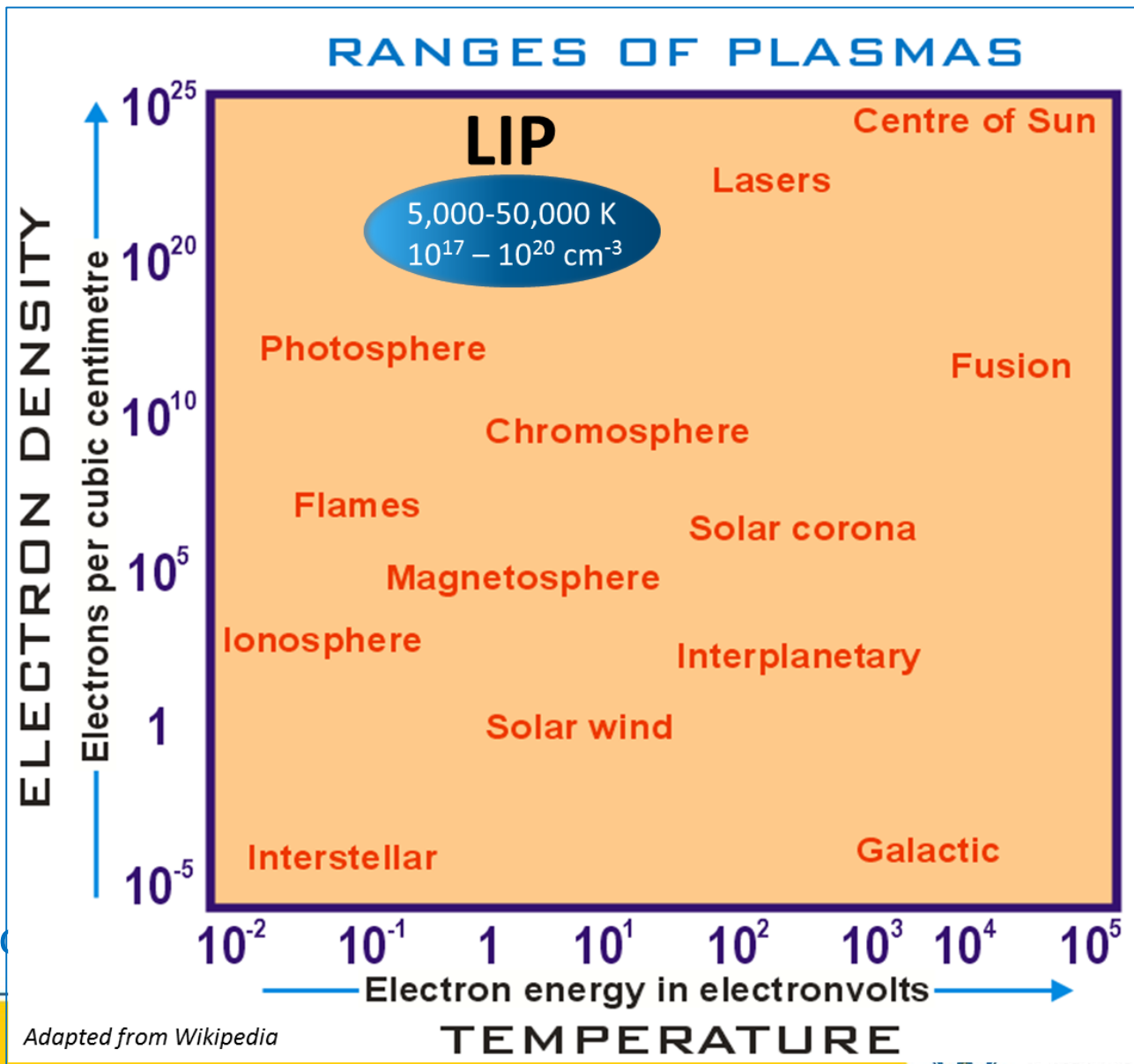
Funding and Acknowledgements

We gratefully acknowledge funding for this project provided by:

- 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 



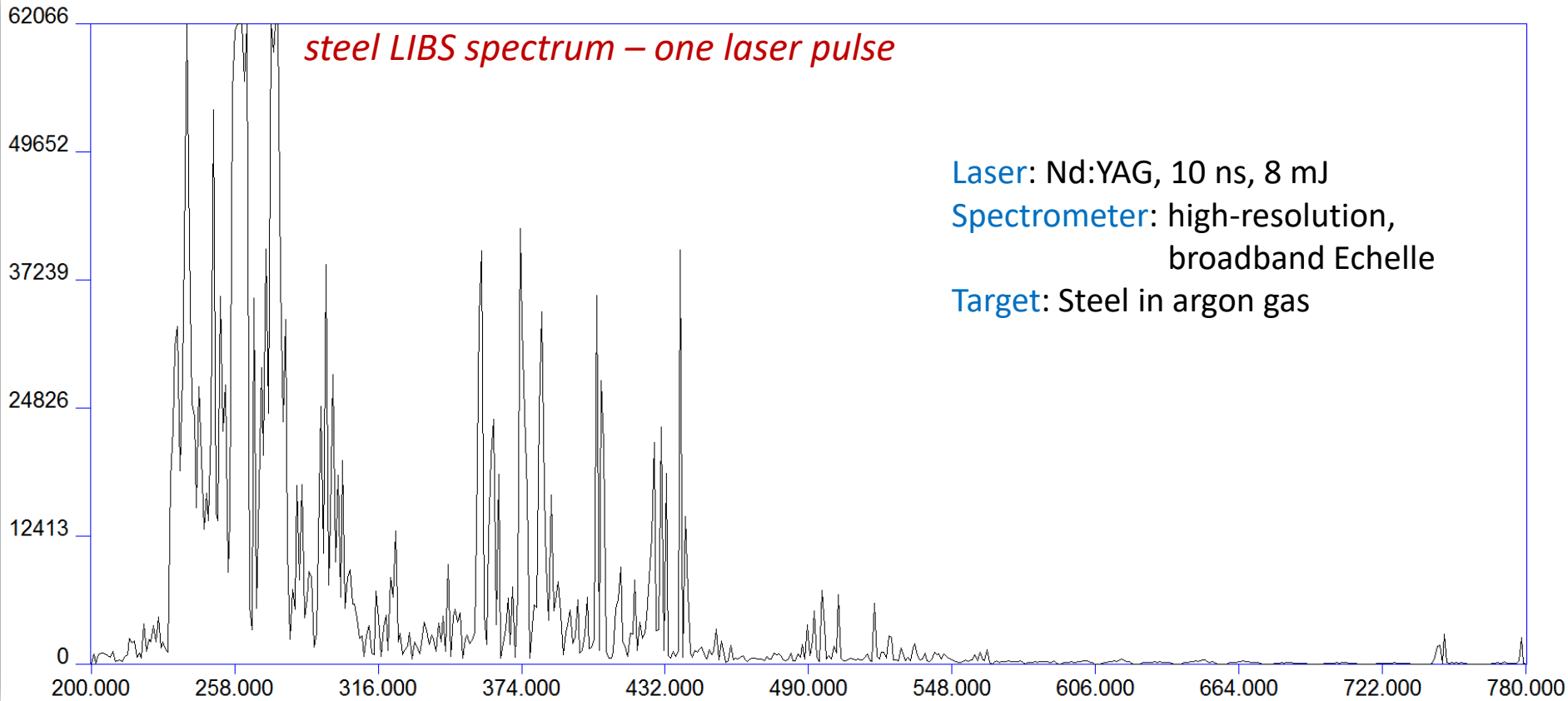
What is “Laser-Induced Breakdown Spectroscopy?”



- Can be
- Thresho

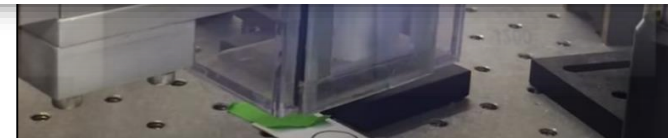
Experimental Apparatus

Optical Tables



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

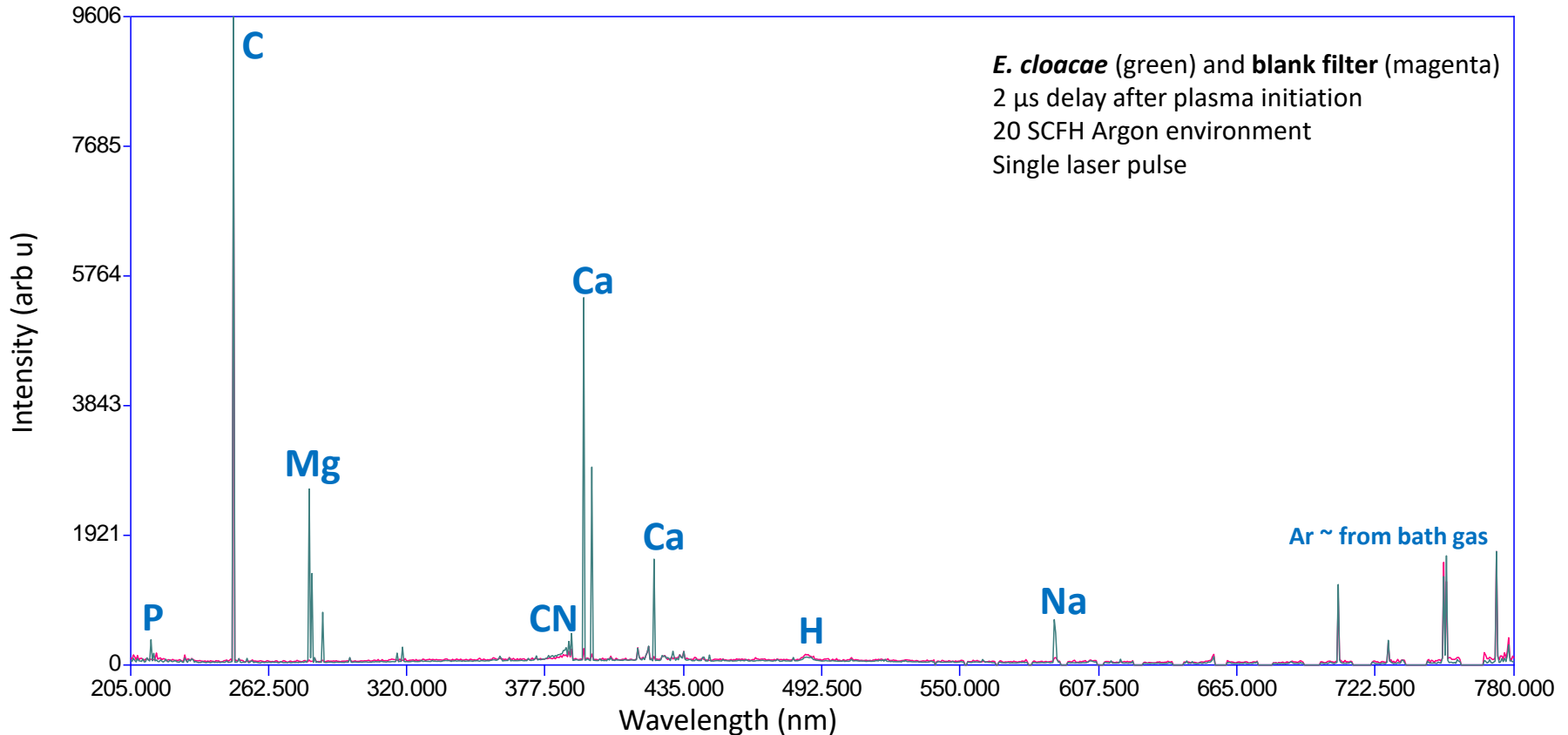
*Steel plasma
in argon*



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Spectrum From Bacteria

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).

Spectrochimica Acta Part B 154 (2019) 50–69



Contents lists available at ScienceDirect

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journal homepage: www.elsevier.com/locate/sab



Invited Review

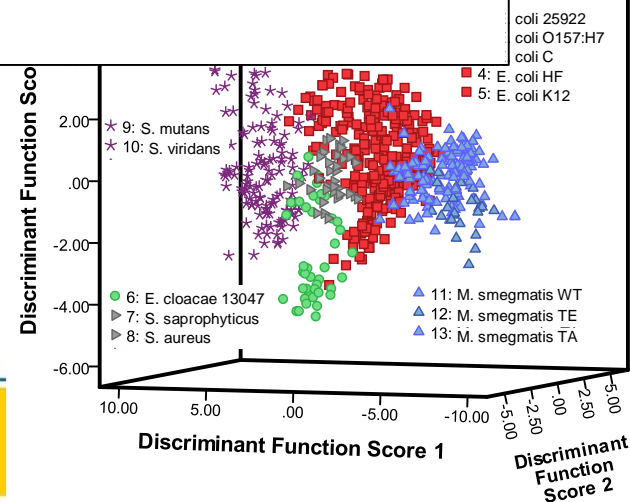
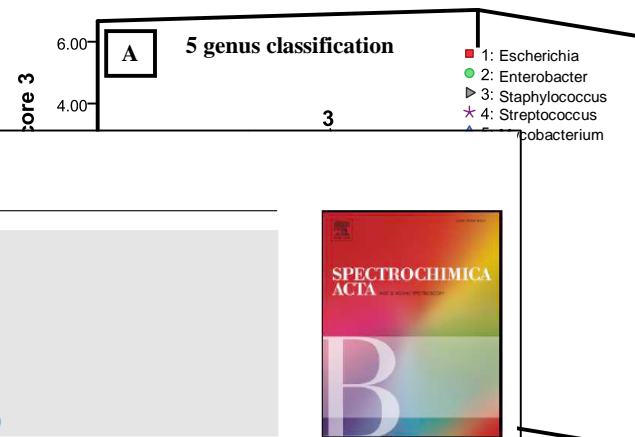
A review of the use of laser-induced breakdown spectroscopy for bacterial classification, quantification, and identification

Steven J. Rehse*

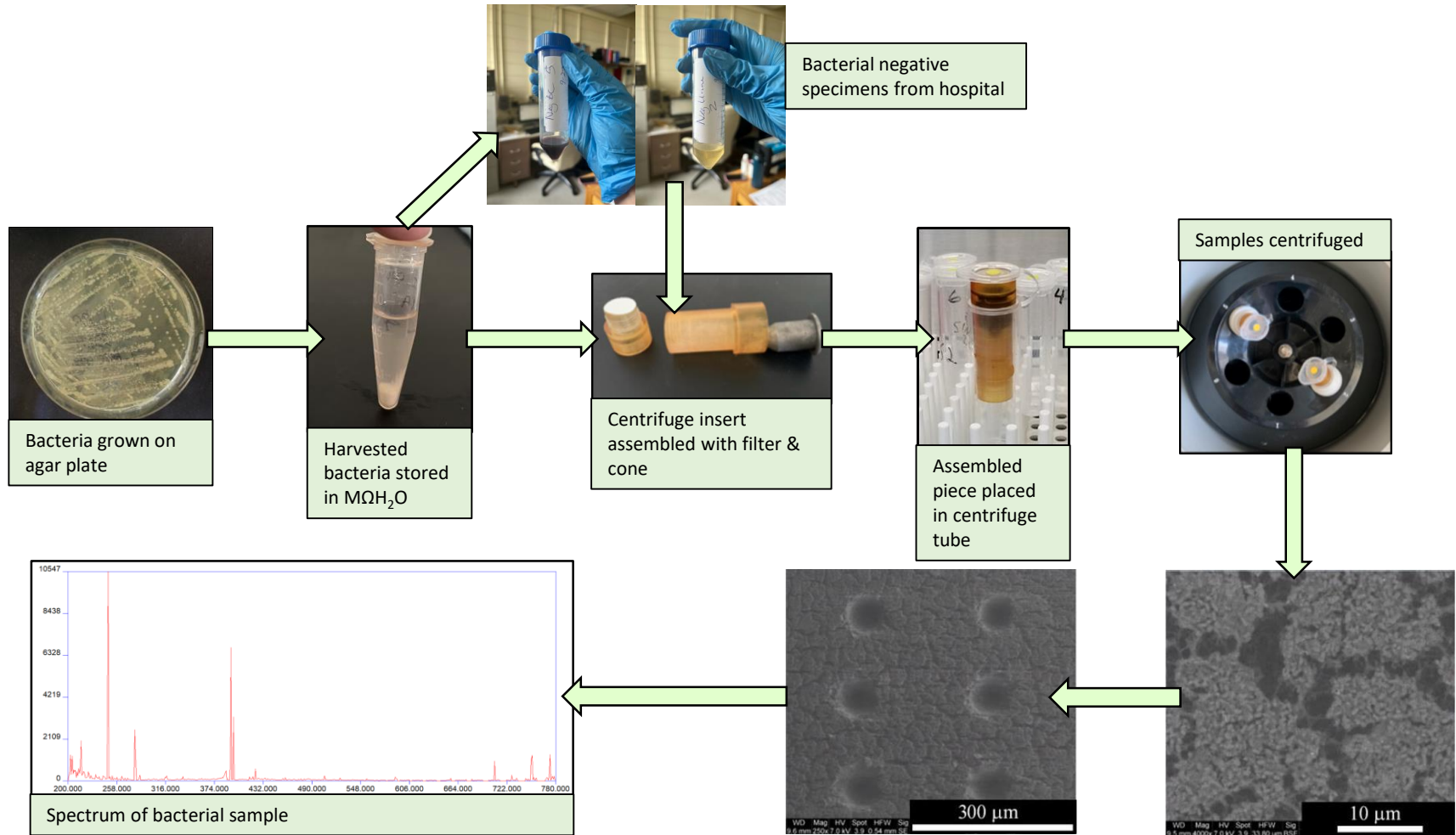
University of Windsor, Department of Physics, Windsor, Ontario N9B 3P4, Canada

	True	False		True	False
Positive	54.05%	0.51%	Positive	86.49%	5.85%
Negative	99.49%	45.95%	Negative	94.15%	13.51%
STREPTOCOCCUS	True	False	STREPTOCOCCUS	True	False
Positive	95.59%	1.02%	Positive	99.26%	13.32%
Negative	98.98%	4.41%	Negative	88.68%	0.74%
MYCOBACTERIUM	True	False	MYCOBACTERIUM	True	False
Positive	88.31%	1.06%	Positive	96.10%	4.08%
Negative	98.94%	11.69%	Negative	95.92%	3.90%

DFA: Sensitivity: $91.37 \pm 16.39 \%$ Specificity: $97.46 \pm 9.35 \%$
PLSDA: Sensitivity: $93.13 \pm 10.25 \%$ Specificity: $90.60 \pm 21.33 \%$

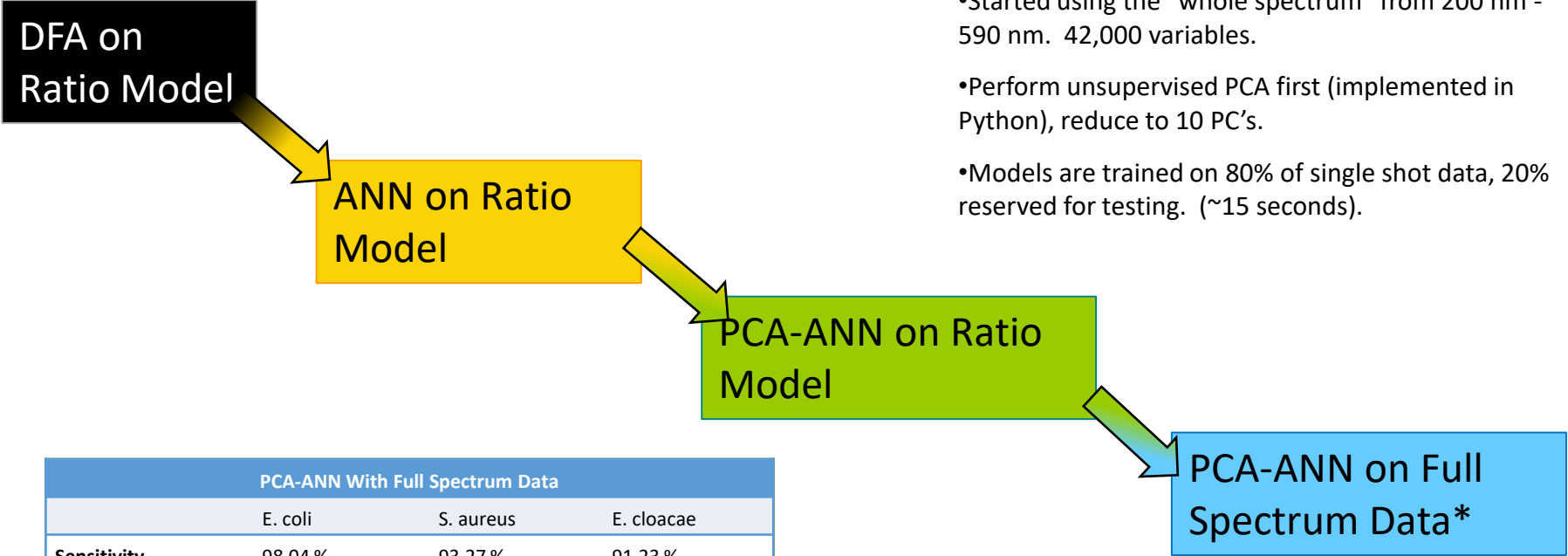


Methodology – Bacterial Growth & Sample Prep



Diagnosing Species with Machine Learning

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



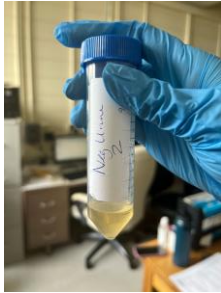
- Started using the “whole spectrum” from 200 nm - 590 nm. 42,000 variables.
- Perform unsupervised PCA first (implemented in Python), reduce to 10 PC’s.
- Models are trained on 80% of single shot data, 20% reserved for testing. (~15 seconds).

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 %

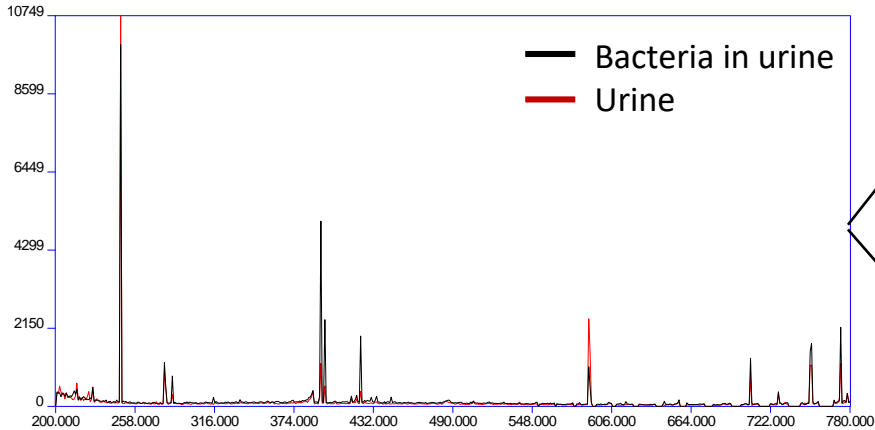
*The full spectrum spans 200 nm – 760 nm, but no lines of interest > 590 nm



Diagnosing Infection (Detecting Bacteria) in Urine



Bacterial-negative specimens obtained from Windsor Regional Hospital

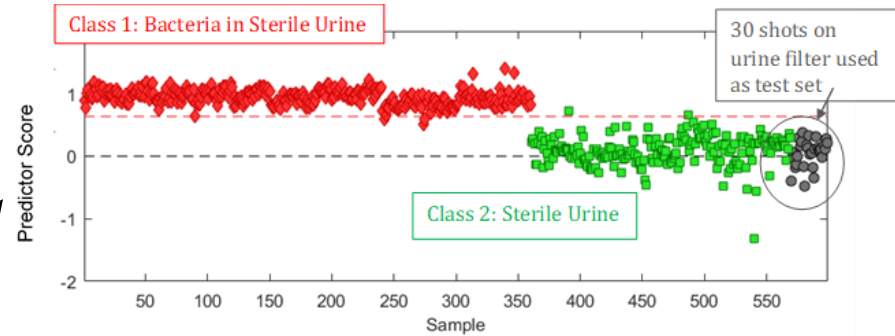


- 360 spectra of bacteria in urine
 - 3 species, four filters of each
- 240 spectra of sterile urine
 - 8 filters

DETECTION IN URINE

98.9% sensitivity

100% specificity



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

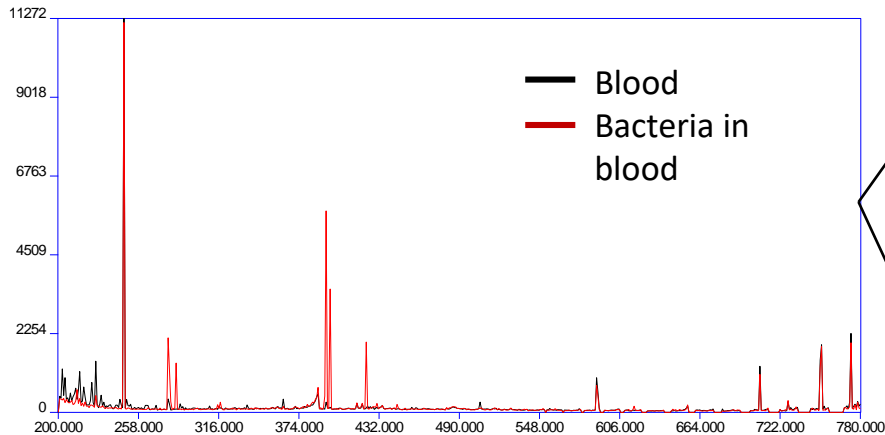
*classification using 80:20 split



Diagnosing Infection (Detecting Bacteria) in Blood



Bacterial-negative specimens obtained from Windsor Regional Hospital

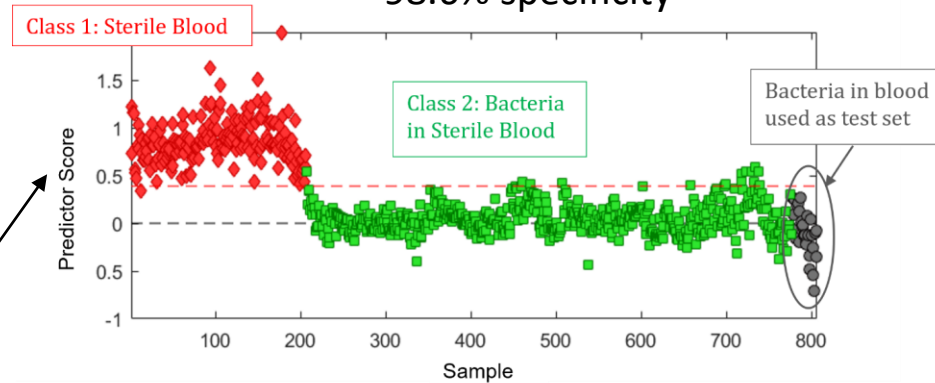


- 600 spectra of bacteria in blood
 - 4 species, five filters of each
- 206 spectra of sterile urine
 - 7 filters

DETECTION IN BLOOD

96.3% sensitivity

98.6% specificity



PCA-ANN With Full Spectrum Data*				
	<i>S. aureus</i>	<i>E. coli</i>	<i>E. cloacae</i>	<i>P. aeruginosa</i>
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 done in both urine and blood
- Improvements need to be made on external validation

average sensitivity of 77.5%

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

average sensitivity of 82.3%

Urine

<i>E. coli</i>	Predicted			
Sample #	<i>S. aureus</i>	<i>E. coli</i>	<i>E. cloacae</i>	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

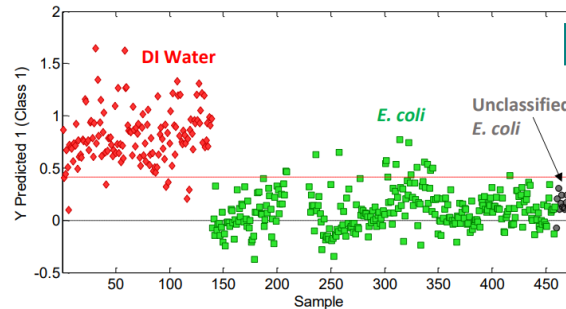
Blood

<i>S. aureus</i>	Predicted				
Sample #	<i>S. aureus</i>	<i>E. coli</i>	<i>E. cloacae</i>	<i>P. aeruginosa</i>	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

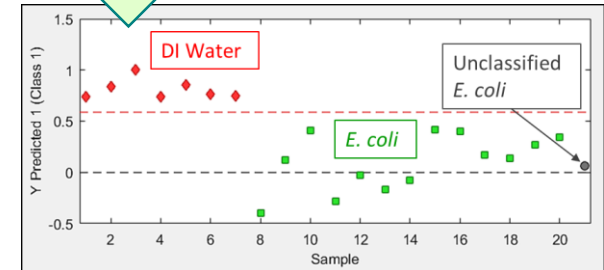


Conclusions

- 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 \approx 30 % (sensitivity), 16% (specificity)



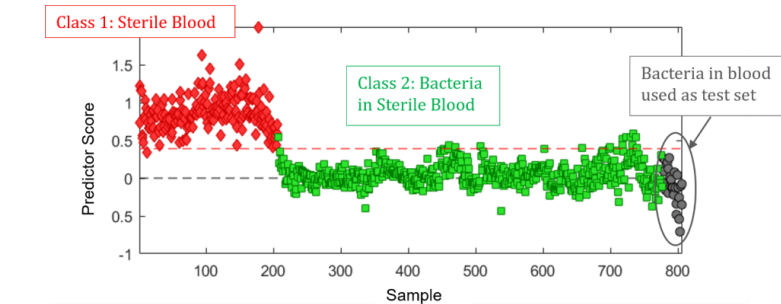
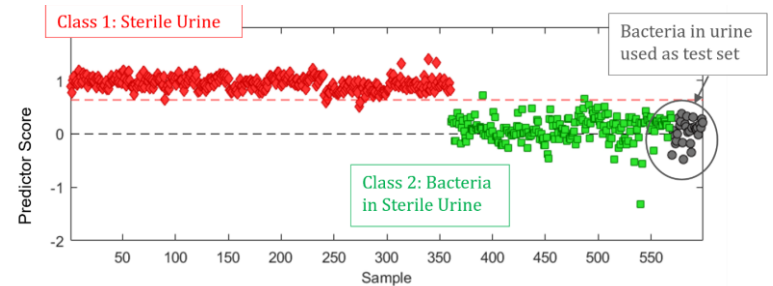
Conclusions

- Future work for PCA-ANN:
 - Improve external validation using PCA-ANN for bacterial species by creating new deposition method
 - Try discrimination of lower concentrations of cells to find limit of identification (LOI)

• 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



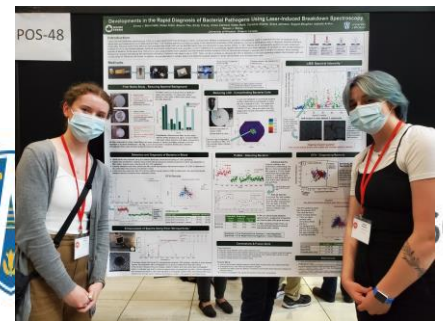
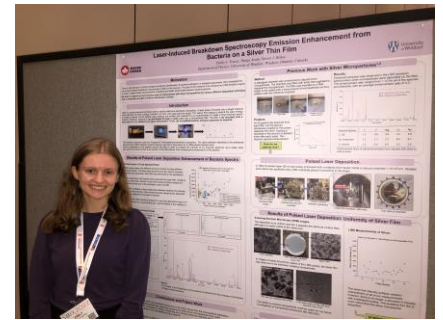
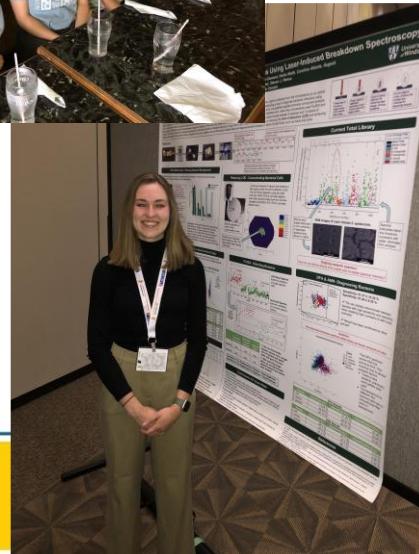
PCA-ANN With Full Spectrum Data (Urine)			
	S. aureus	E. coli	E. cloacae
Sensitivity	100 %	100 %	91.67 %
Specificity	100 %	95.83 %	100 %
Classification Error	0.00 %	2.09 %	4.17 %

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 %



Acknowledgements for the people who did the work...

2022-2023



tor

Thank you!



YouTube

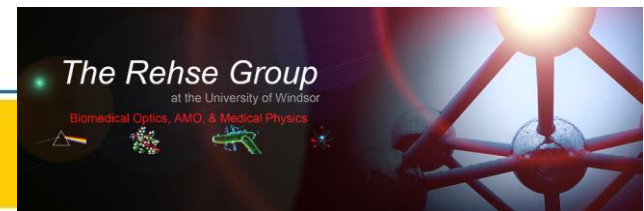
Webpage: www.uwindsor.ca/people/rehse/



Rehse UWindsor Class Channel

@rehseuwindsorclasschannel6484 222 subscribers 52 videos

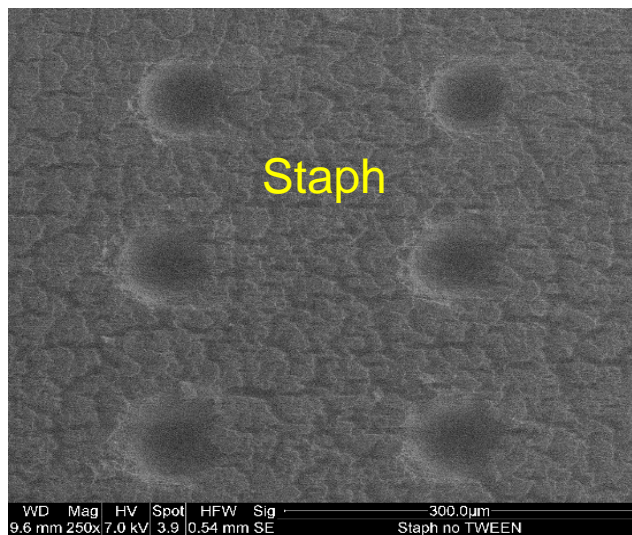
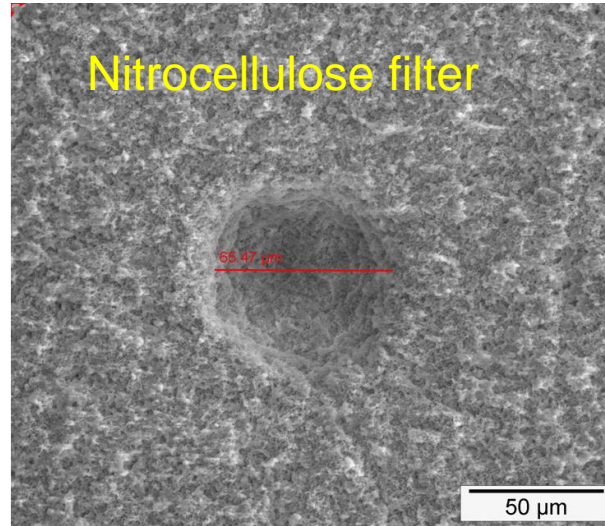
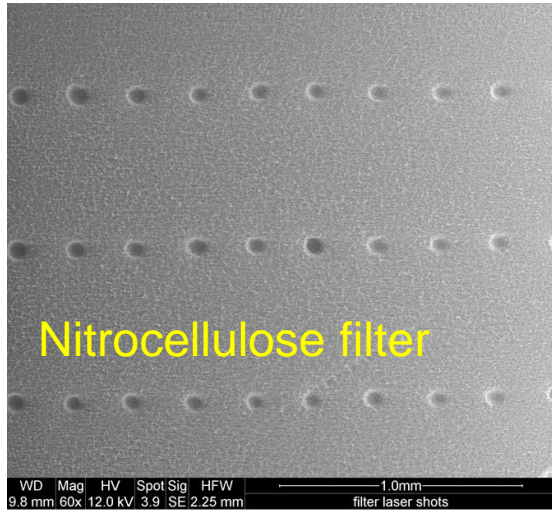
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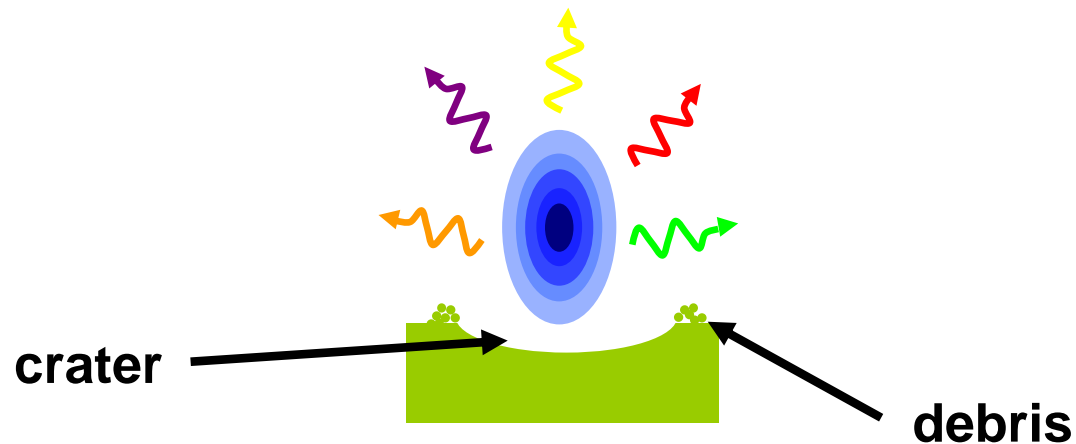
University of Windsor

2) removal of samples mass (ablation)

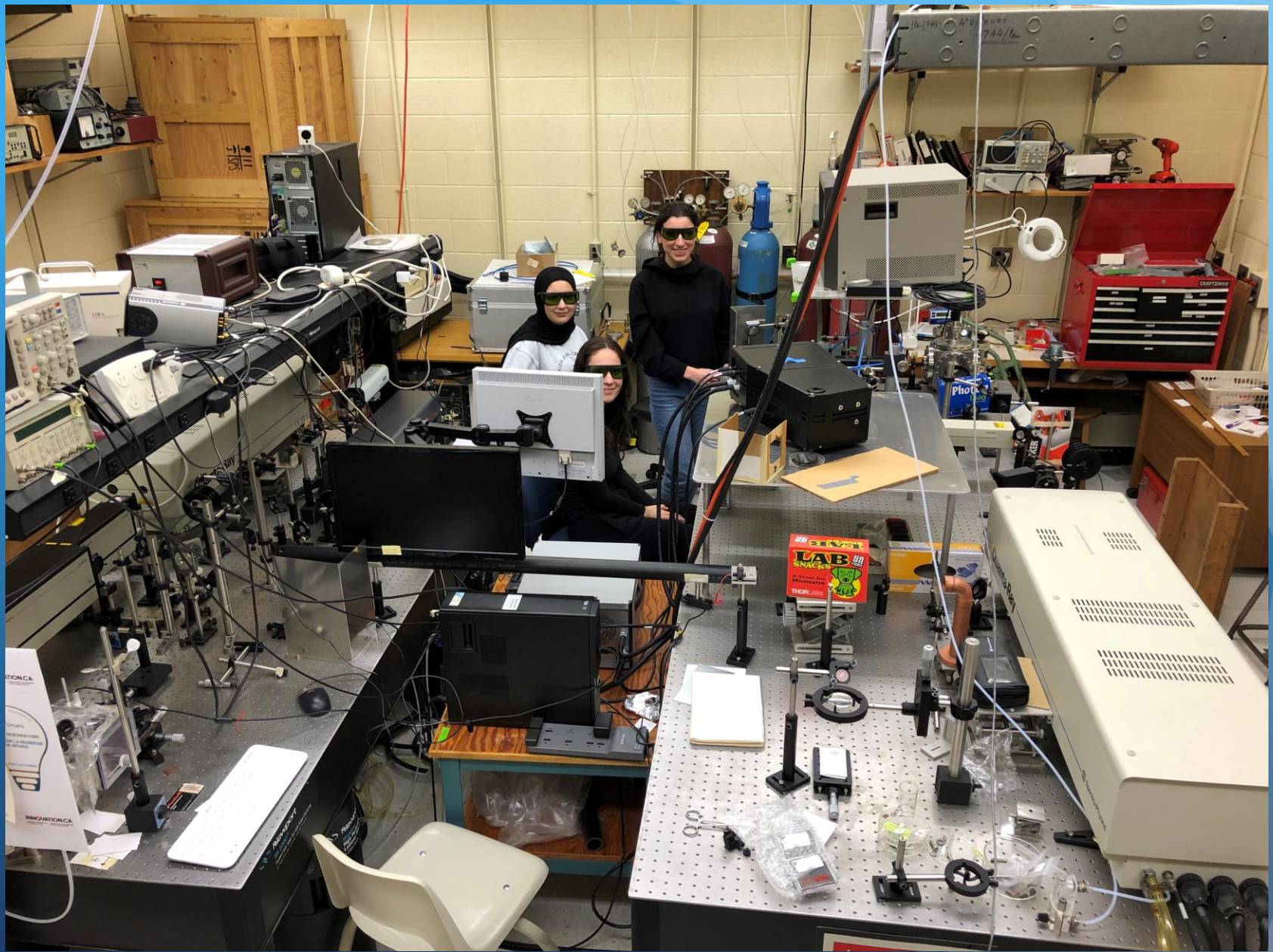


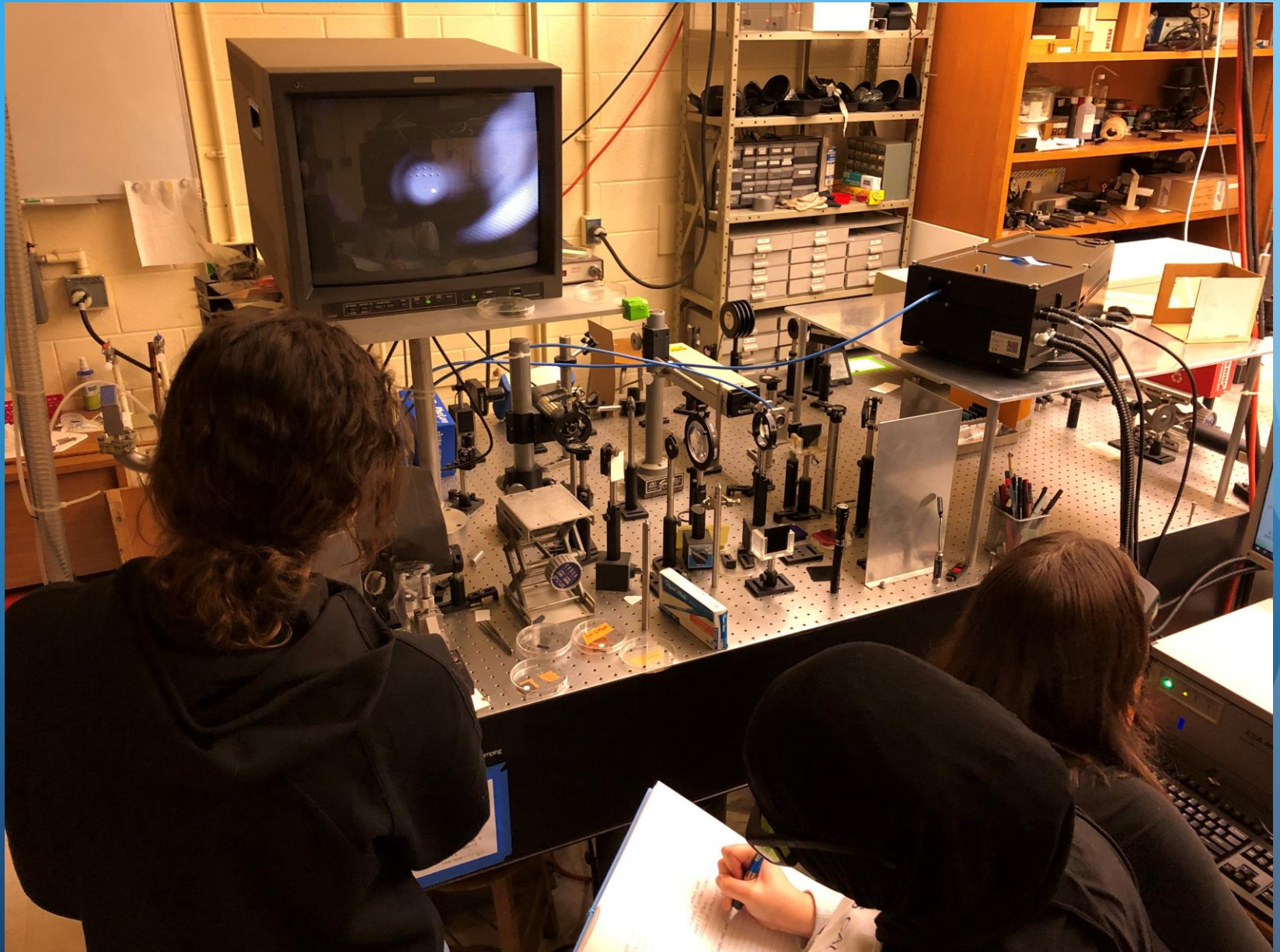
**4) expansion and
element specific
emission (atomic or
ionic)**

**spontaneous emission
as atoms/ions decay to
ground state**

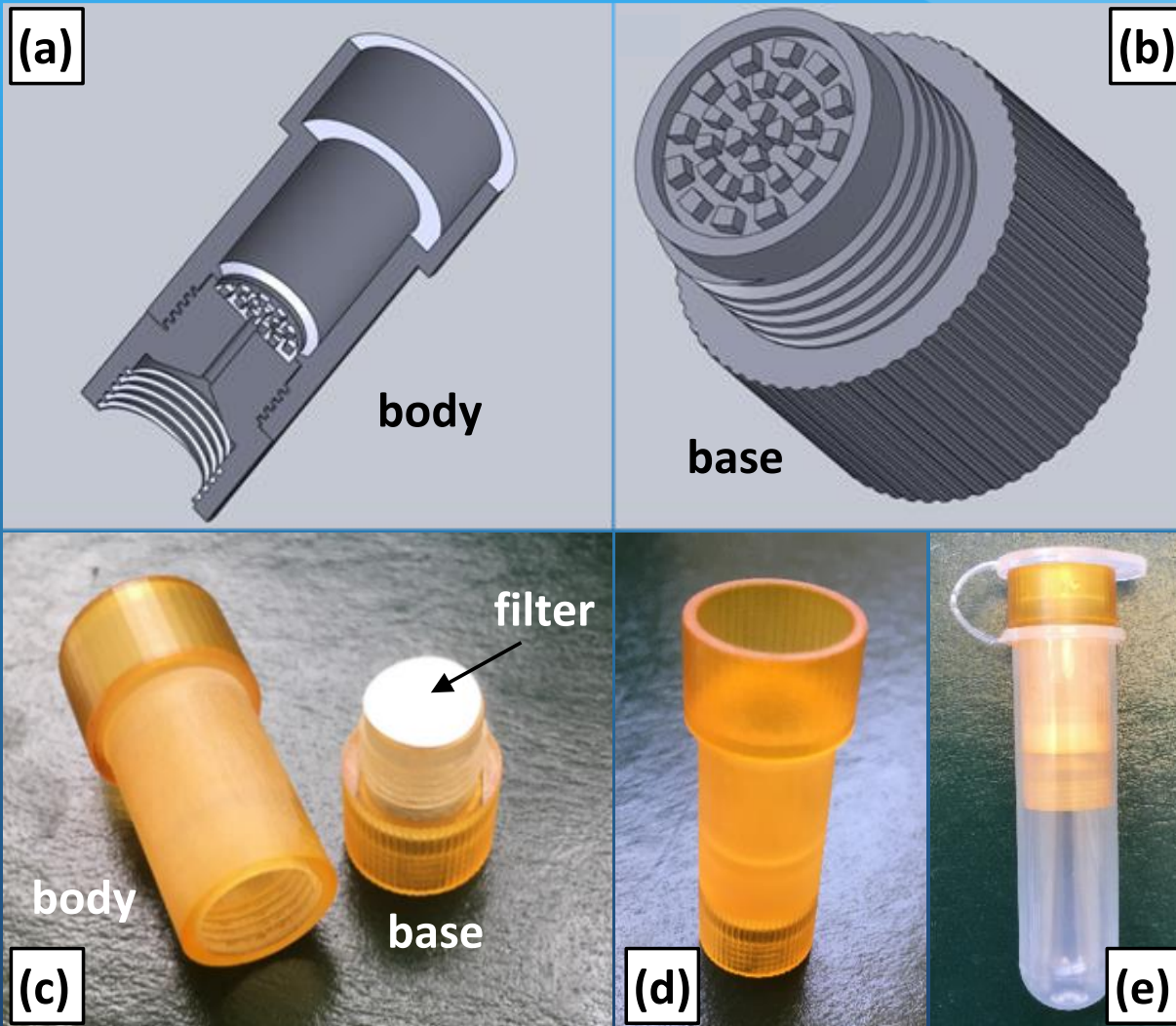


- The dynamic evolution of the plasma plume is then characterized by a fast expansion and subsequent cooling.
- Approximately 1 microsecond after the ablation pulse, spectroscopically narrow atomic/ionic emissions may be identified in the spectrum.



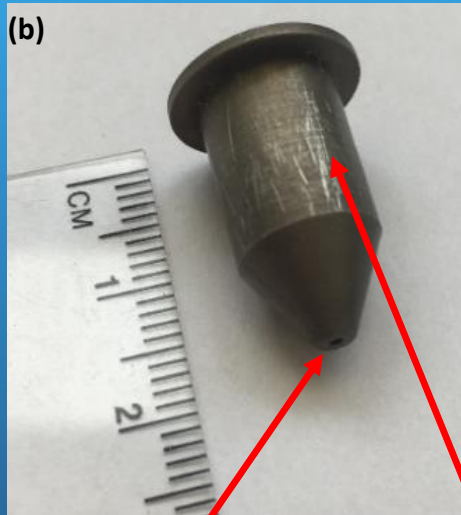


The Centrifuge Insert



Concentrating Bacteria With a Cone

19 mm long Al cone



Centrifuge tube cap presses cone into filter



1 mm hole at apex

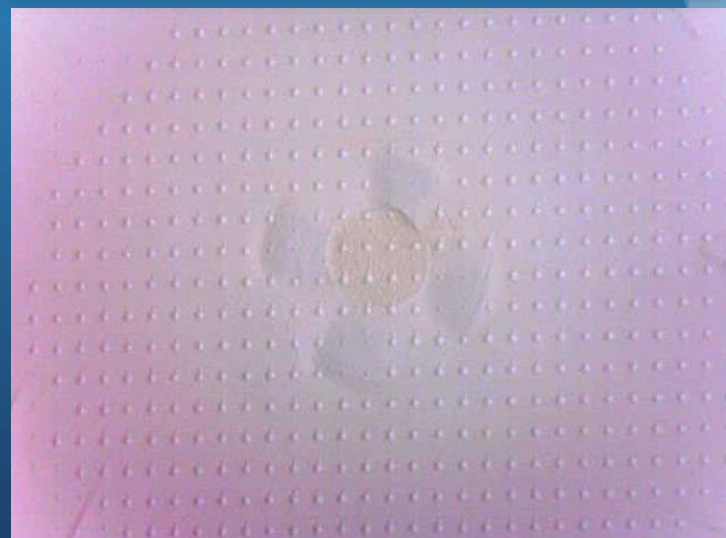
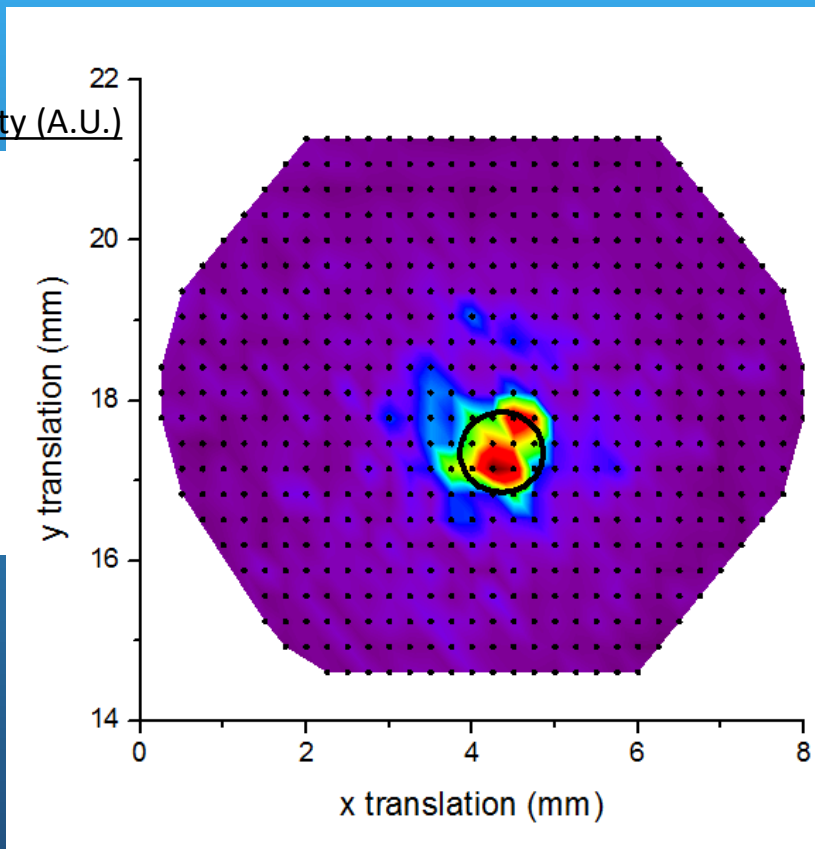
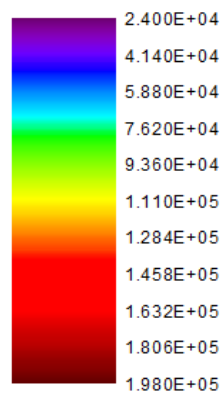
Holds 1 mL of fluid



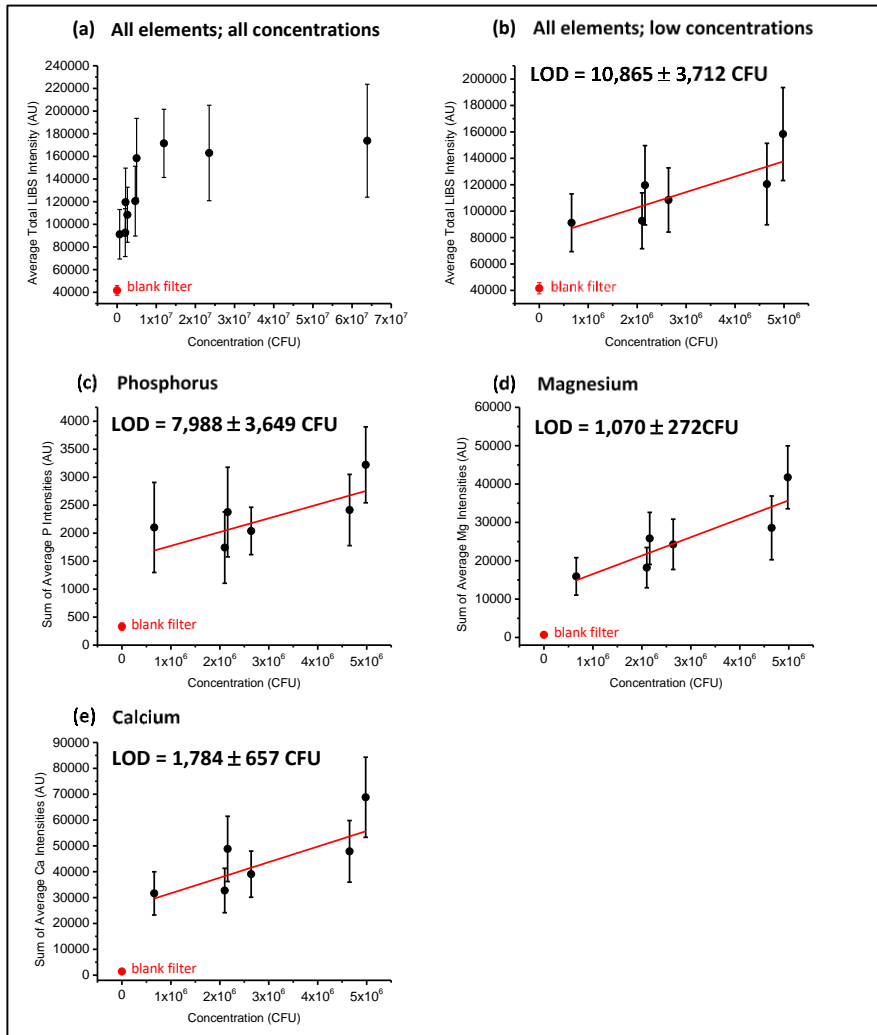
Cone vertex press fit into filter

Shooting Bacteria Concentrated With Cone

Total LIBS intensity (A.U.)



Limits of Detection



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

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

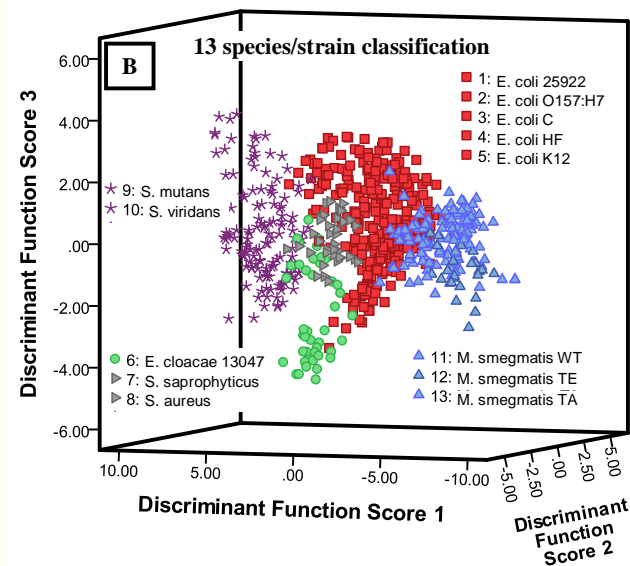
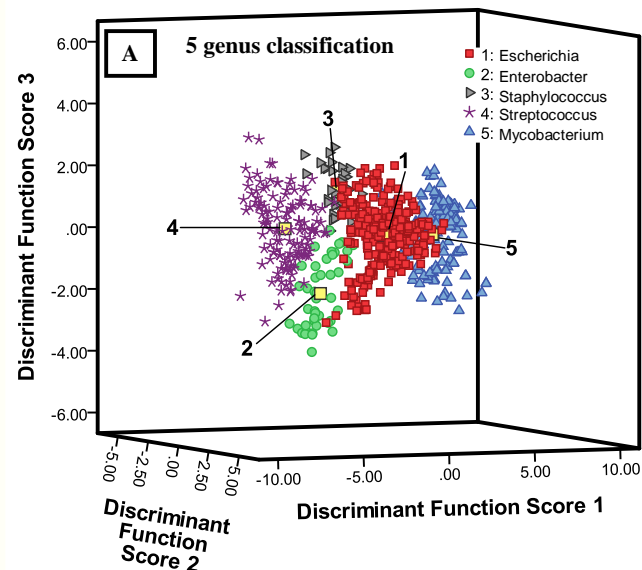
LOD's calculated using only certain elements observed in the LIBS spectra and present in very low concentrations in the filter were even lower:

$1,070 \pm 272$ CFU for magnesium
 $1,784 \pm 657$ 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

<i>E. COLI</i>	True	False
Positive	95.65%	9.17%
Negative	90.83%	4.35%

<i>STAPHYLOCOCCUS</i>	True	False
Positive	54.05%	0.51%
Negative	99.49%	45.95%

<i>STREPTOCOCCUS</i>	True	False
Positive	95.59%	1.02%
Negative	98.98%	4.41%

<i>MYCOBACTERIUM</i>	True	False
Positive	88.31%	1.06%
Negative	98.94%	11.69%

DFA

<i>E. COLI</i>	True	False
Positive	89.63%	15.95%
Negative	84.05%	10.37%

<i>STAPHYLOCOCCUS</i>	True	False
Positive	86.49%	5.85%
Negative	94.15%	13.51%

<i>STREPTOCOCCUS</i>	True	False
Positive	99.26%	13.32%
Negative	88.68%	0.74%

<i>MYCOBACTERIUM</i>	True	False
Positive	96.10%	4.08%
Negative	95.92%	3.90%

DFA: Sensitivity: $91.37 \pm 16.39 \%$ Specificity: $97.46 \pm 9.35 \%$
PLSDA: Sensitivity: $93.13 \pm 10.25 \%$ Specificity: $90.60 \pm 21.33 \%$

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...

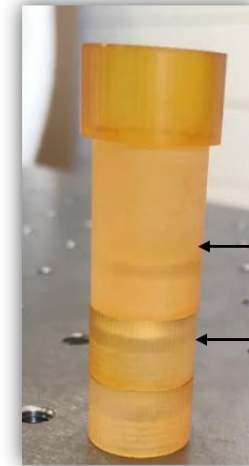
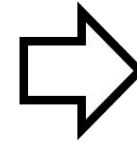
Results: We have already demonstrated...

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



5 μm filter



0.45 μm filter