Recent Advances in the Use of Laser-Induced Breakdown Spectroscopy to Classify Pathogens in Clinical Specimens

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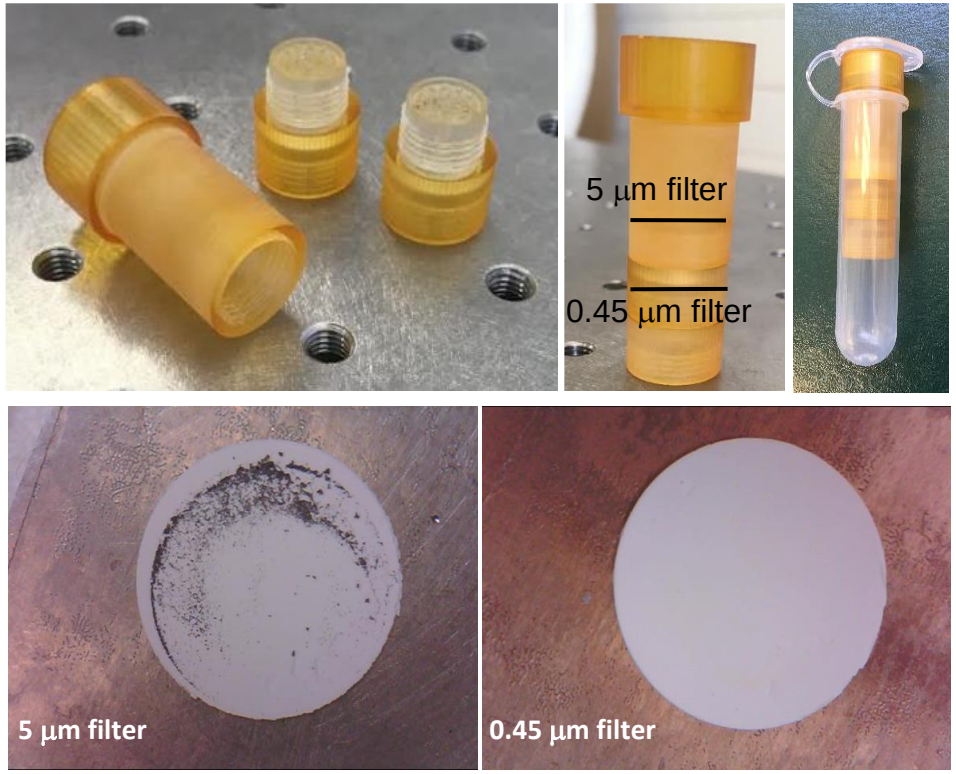
University of Windsor

History and Motivation

- Since I have been at the University of Windsor, we have invented a new way to analyze bacterial cells in liquid suspension.
- This method was used for all the studies described in this talk.

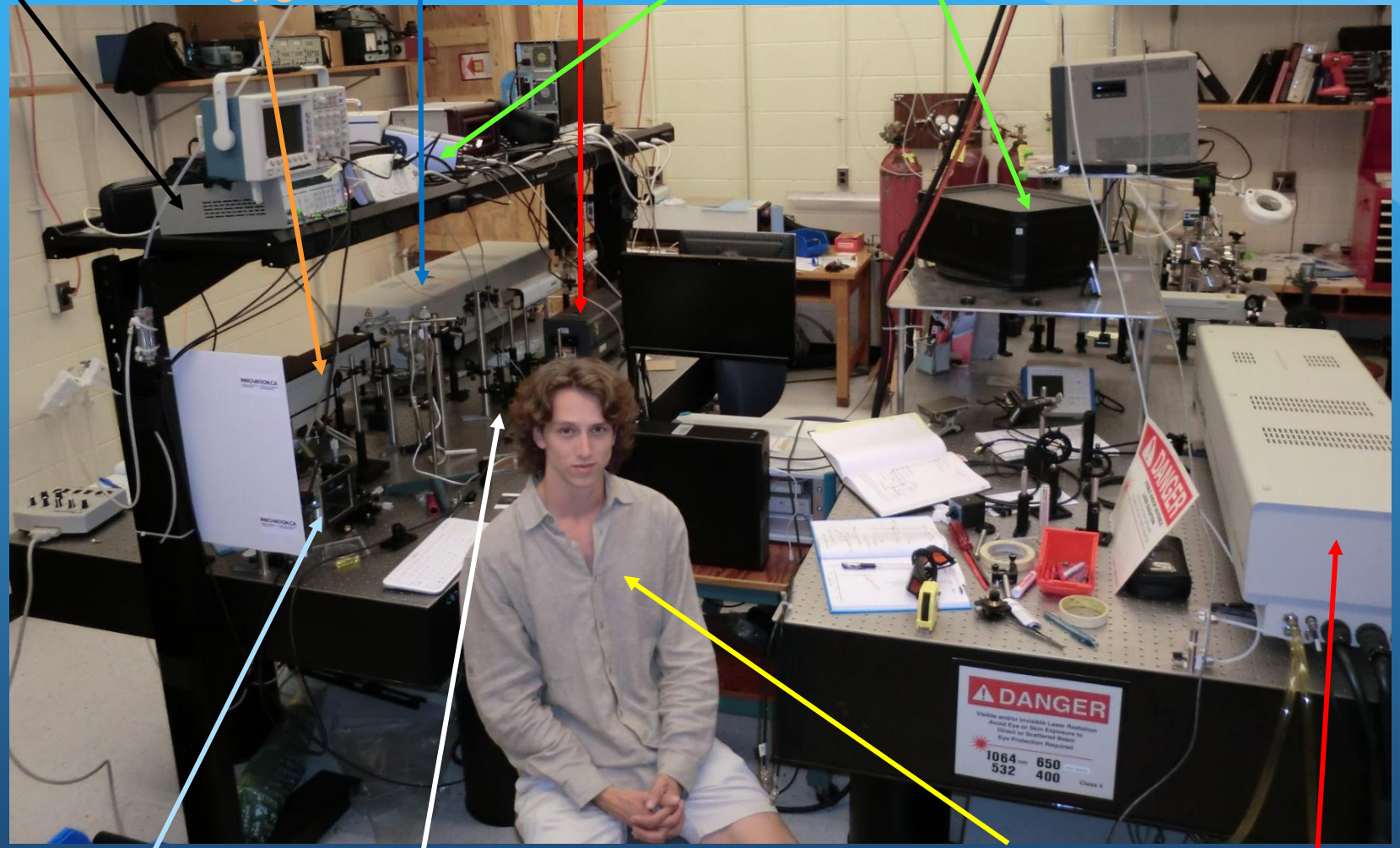
Sample Centrifugation

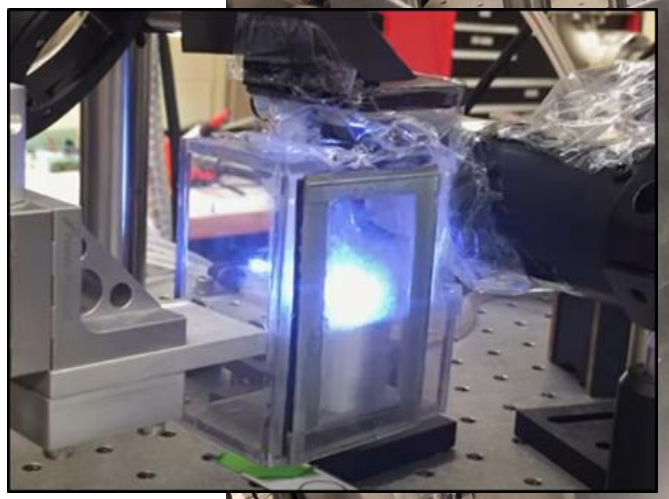


- We culture our own bacteria

- 3D printed “centrifuge insert”
- Nitrocellulose filters (Millipore. 0.45 and 0.22 μm)
- 1 mL of bacterial suspension centrifuged at 5000 rpm
- Filter removed, bacteria on filter ablated

- Second base constructed to allow dual stage centrifugation


LIBS Table 1
1064 nm LIBS laser
355 nm OPO pump
Echelle spectrometer/camera
LIBS Table 2
Timing control
OPO
DANGER
Argon chamber
Photodiode for observing pulse timing
Student to run the whole thing
1064 nm LIBS laser



IR Nd:YAG laser

Echelle spectrometer

microscope assembly

sample chamber (w/ purge gas)

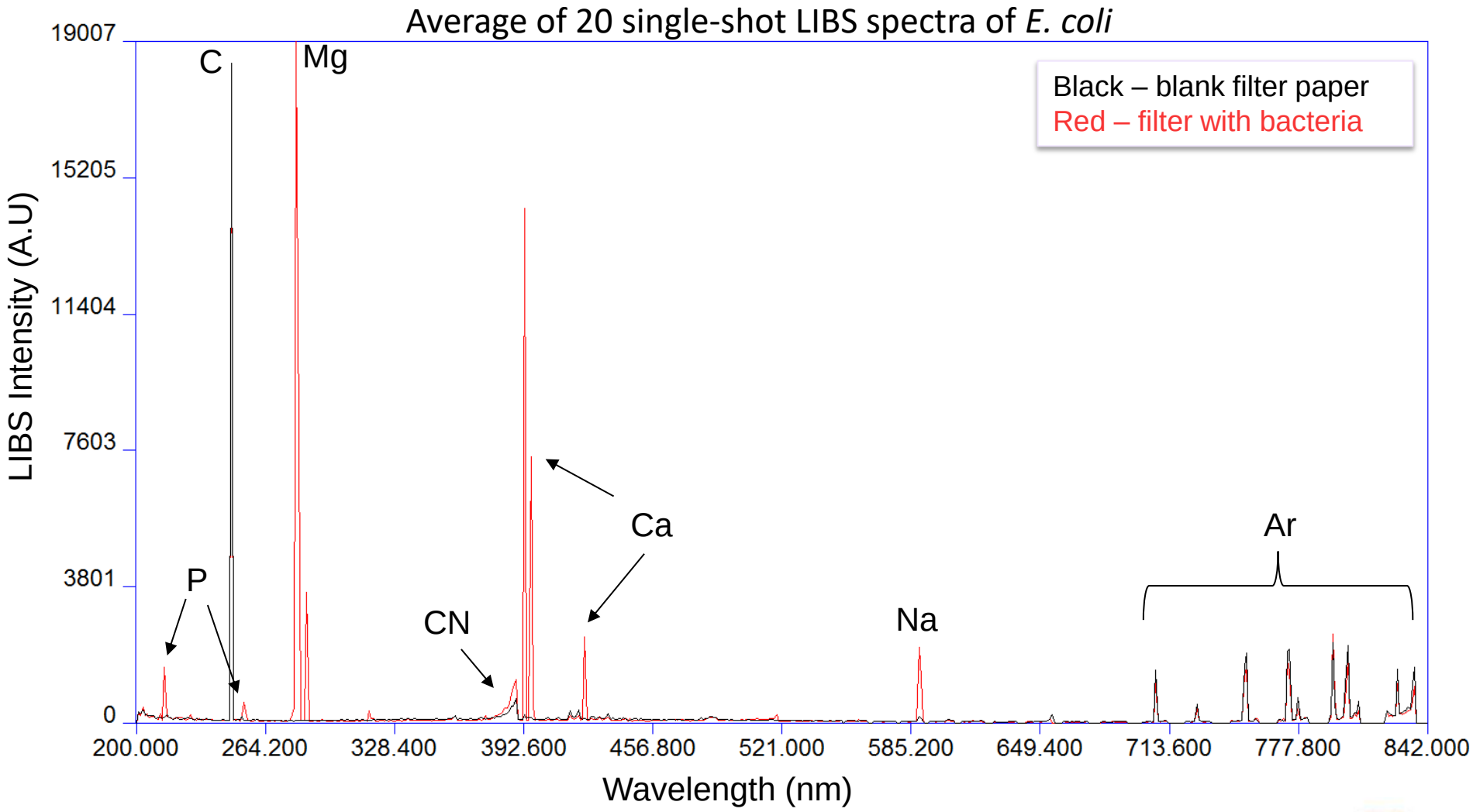
Newport

RS 2000™

Hole Table Top with Tuned Damping

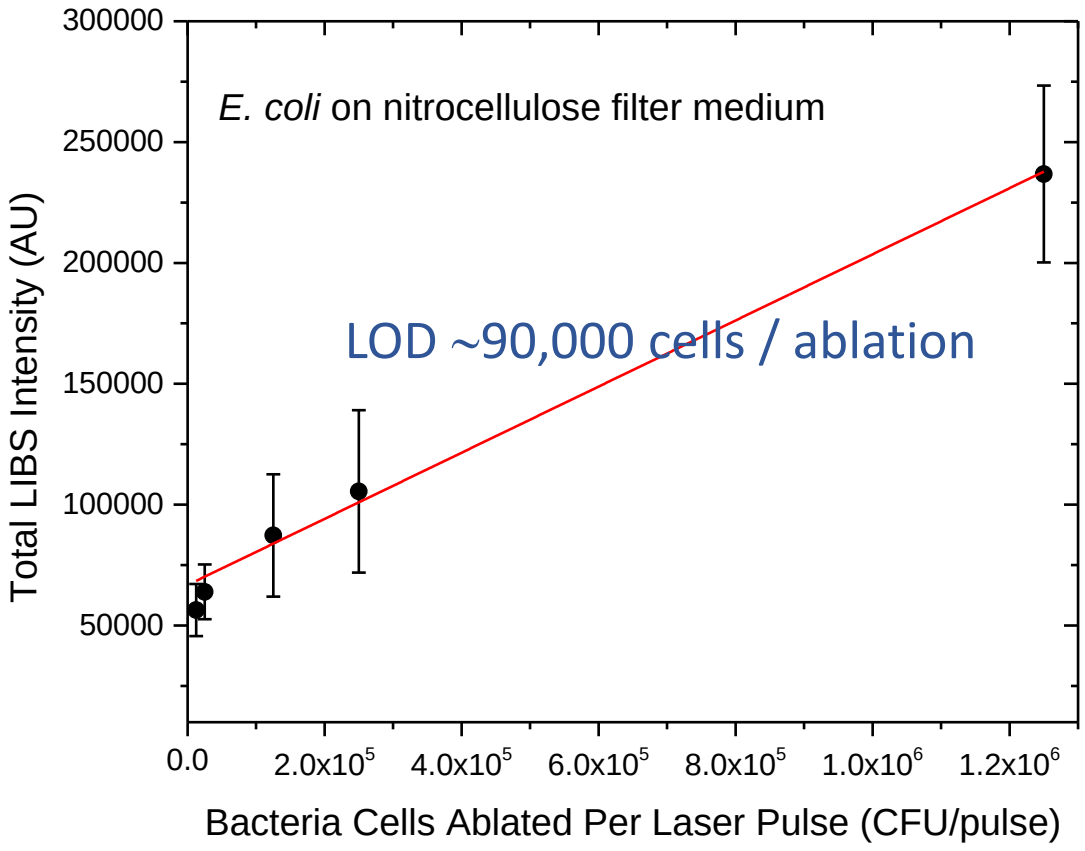
of Windsor

Sample Centrifugation



- 1064 nm, 10 ns laser
- 8 mJ/pulse
- LLA ESA3000 w/ICCD
- τ_{delay} = 2 μs
- τ_{width} = 20 μs
- argon purge
- single shot spectra (all bacteria ablated after one shot)

Sample Centrifugation



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- argon purge
- single shot spectra (all bacteria ablated after one shot)

Analytical note Spectrochimica Acta Part B 158 (2019) 105629

A simple and efficient centrifugation filtration method for bacterial concentration and isolation prior to testing liquid specimens with laser-induced breakdown spectroscopy☆

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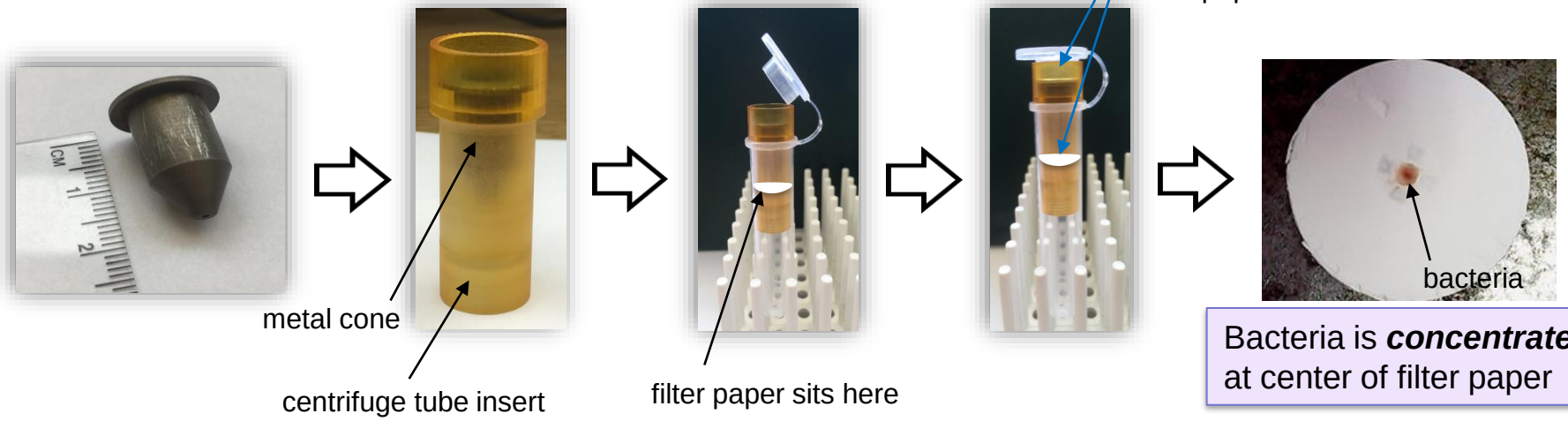
University of Windsor

Sample Concentration

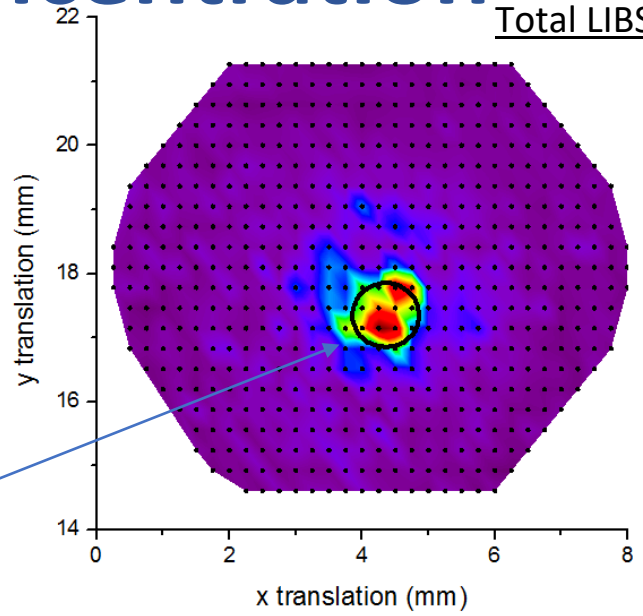
Method:



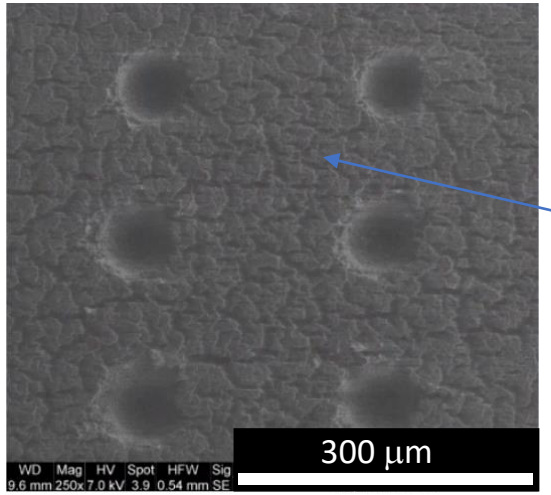
Bacteria is ***concentrated*** at center of filter paper

Sample Concentration

Total LIBS intensity (A.U.)

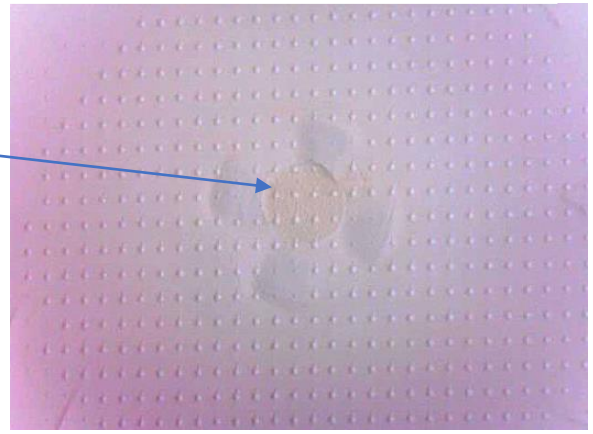


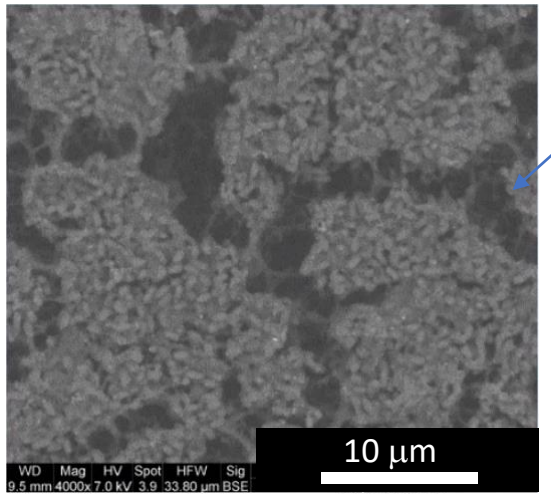
Cone nozzle ~1 mm



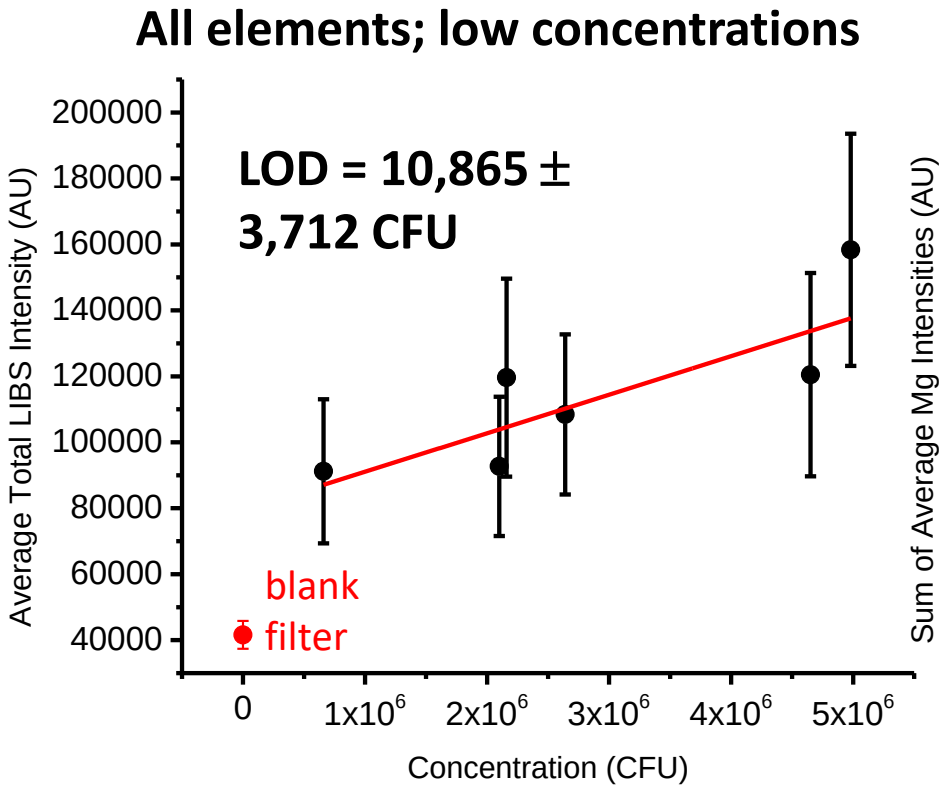
Irregular deposition could be problematic

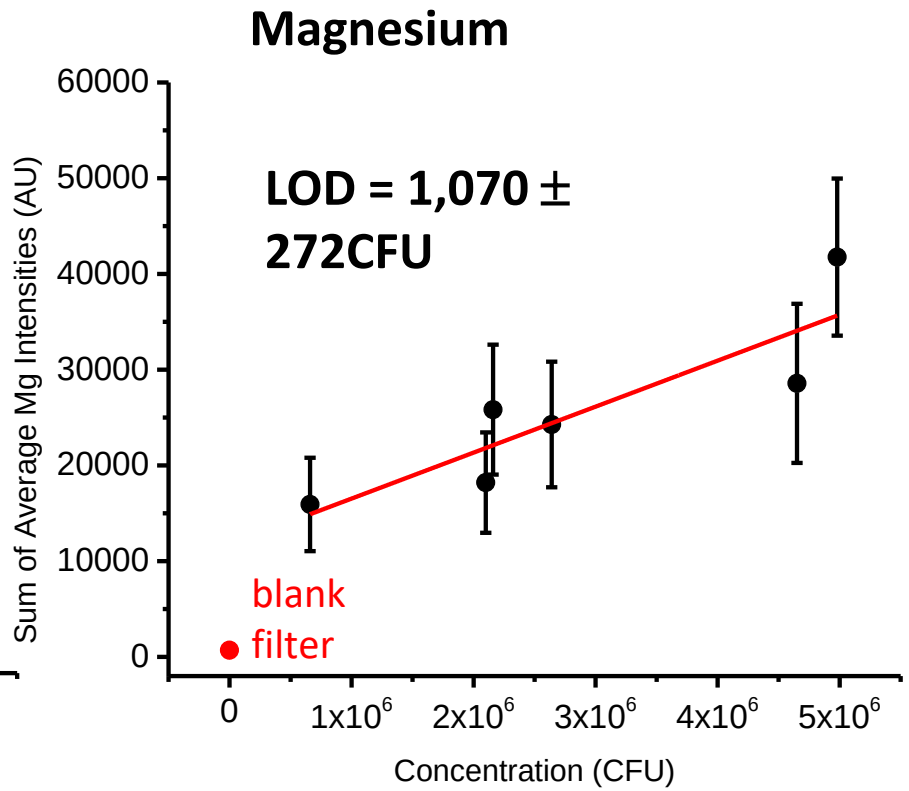
We can reliably acquire 20-30 shots per deposition





Sample Concentration





Spectrochimica Acta Part B 157 (2019) 68–75

Concentration of bacterial specimens during centrifugation prior to laser-induced breakdown spectroscopy analysis☆

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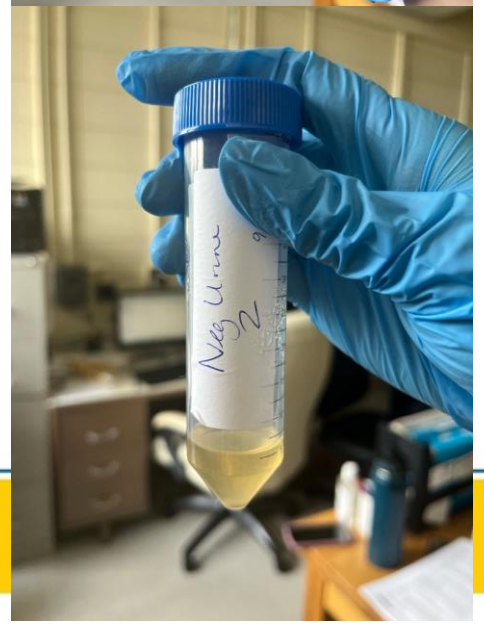
University of Windsor

Recent Progress

i. Biofluids (blood and urine) from hospital
ii. Full-spectrum chemometrics utilizing a PCA-ANN
iii. Use of silver to enhance plasma emission

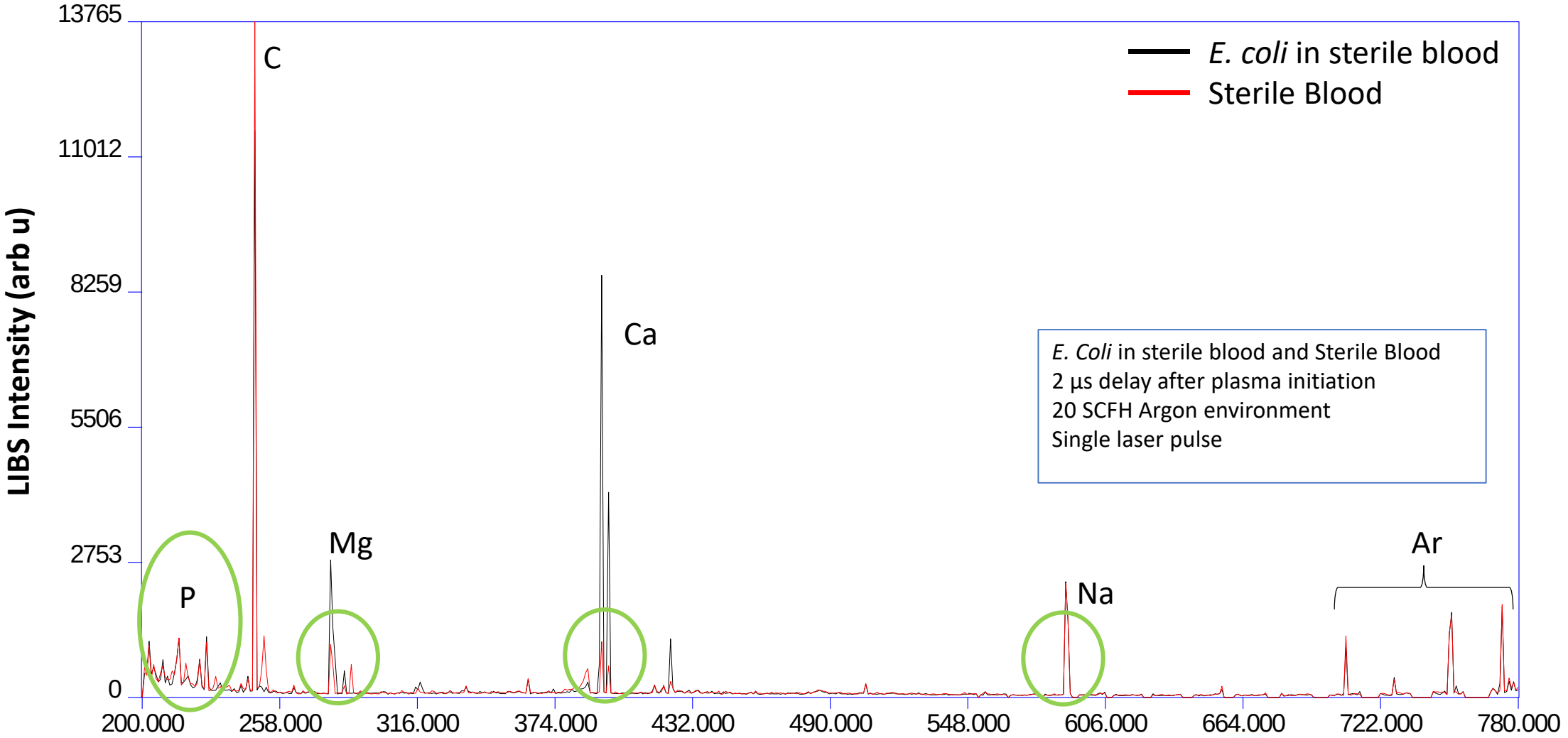
Recent Progress (i): Blood & Urine

After testing in hospital pathology lab, bacterial negative, specimens sent to us

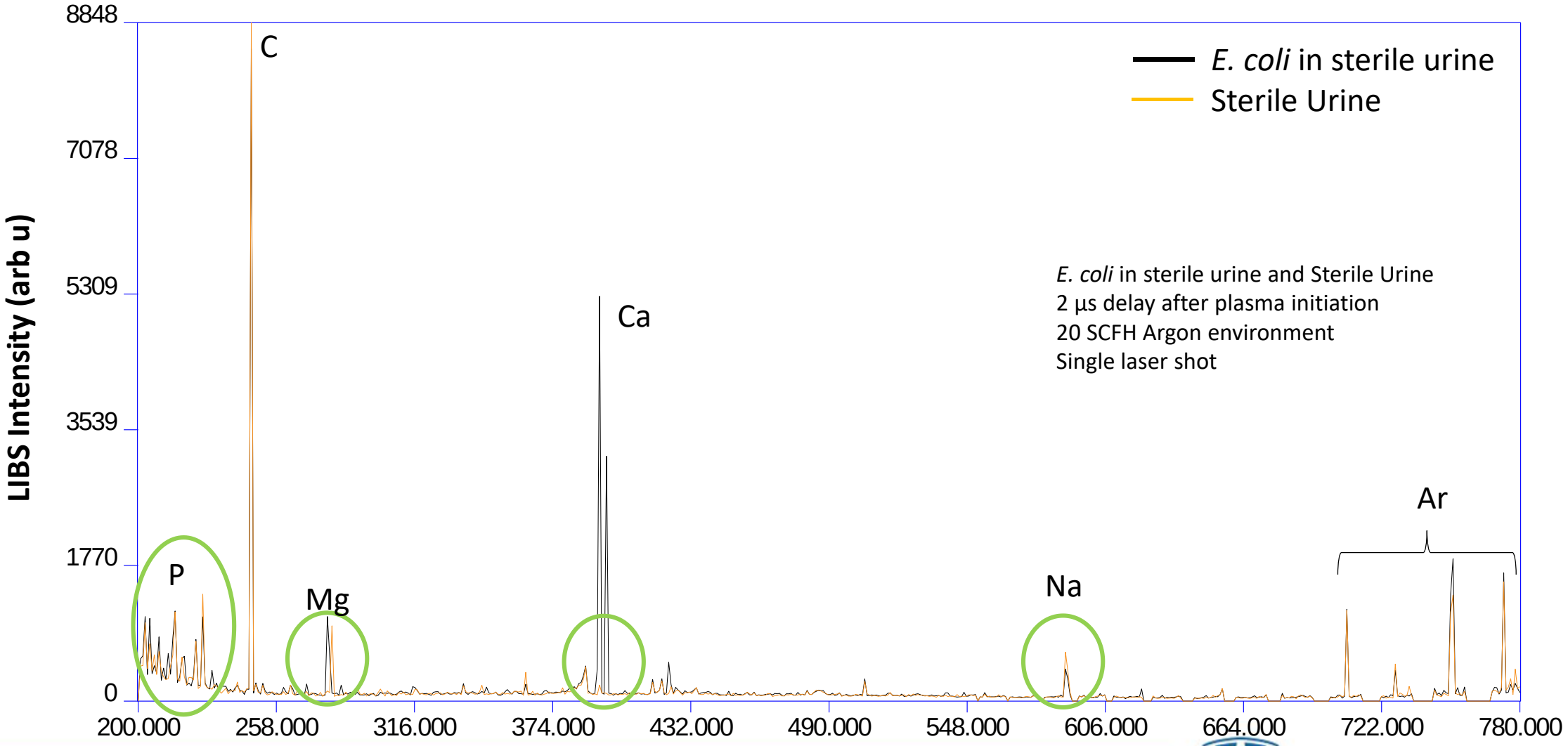


We “spike” the nominally sterile specimens with trace amounts of known bacteria (e.g. *E. coli*) to simulate sepsis and UTI.

Recent Progress (i): Blood & Urine



Recent Progress (i): Blood & Urine

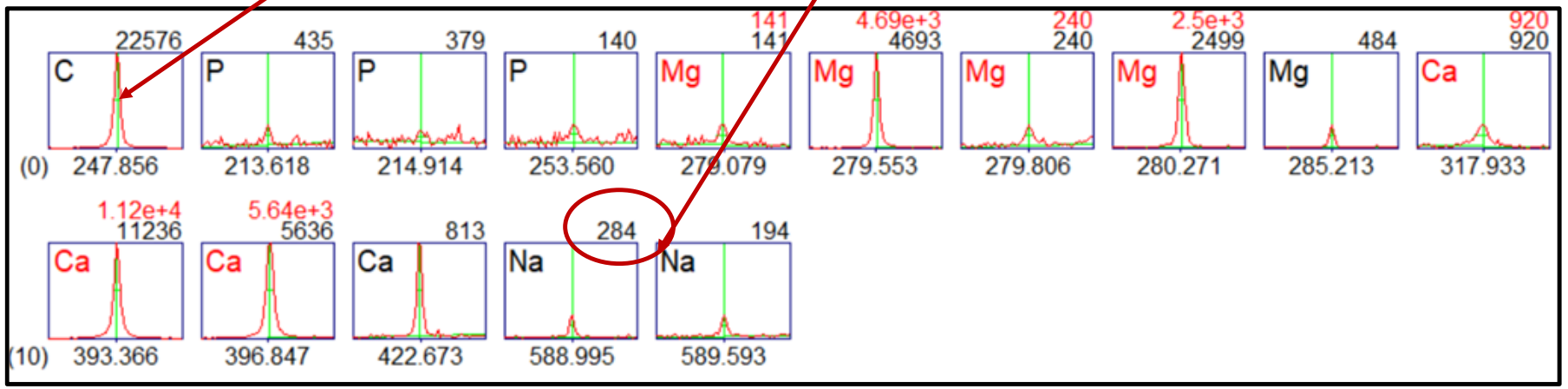


Recent Progress (i): Blood & Urine

Variable down-selection. Ratio-model 2.5: 15 line areas, 92 ratios = 107 variables

Strong emission of carbon line comes from filter

Emission intensity is the integrated area under the curve

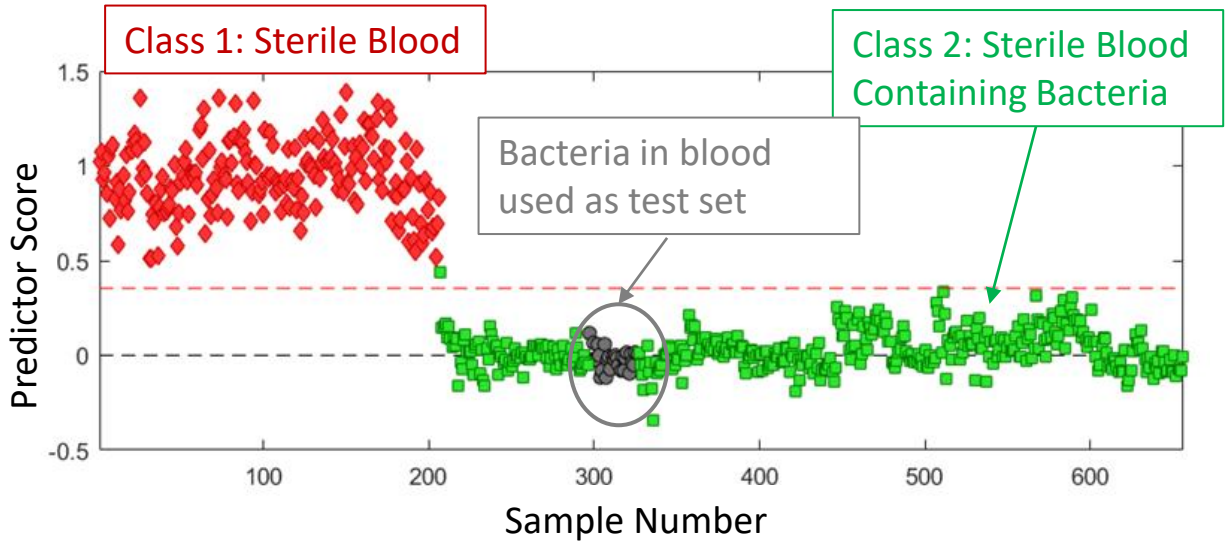


Phosphorus lines are highly indicative of bacteria. Na, Ca, and Mg also indicative of bacteria. All of these elements exist in the bacterial cell membrane.

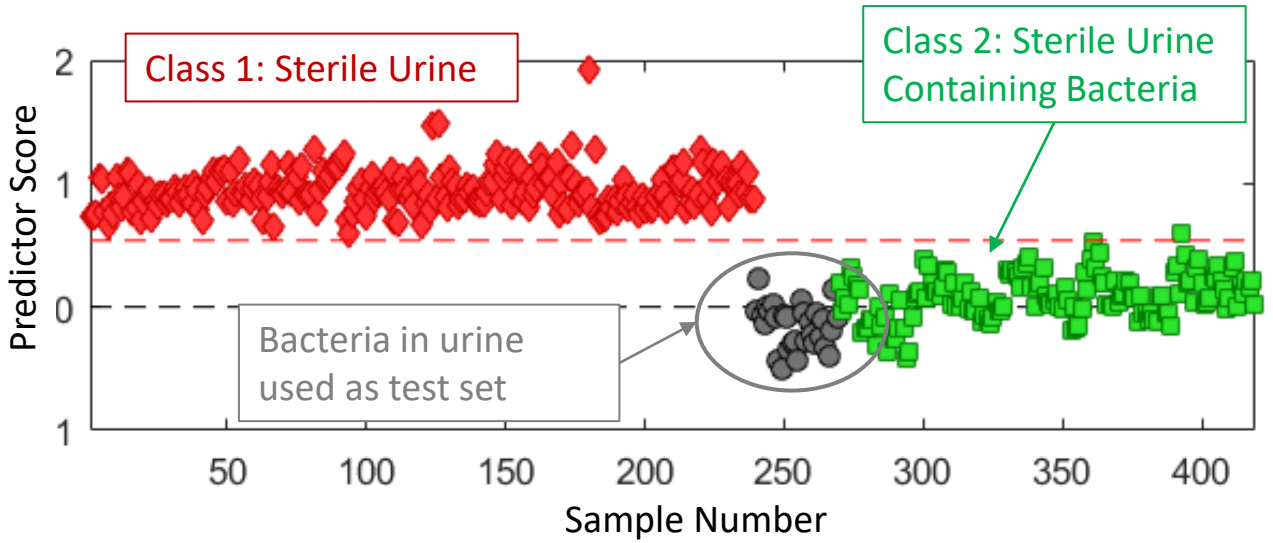
Recent Progress (i): Blood & Urine

- A **partial least squares discriminant analysis (PLSDA)** test was conducted using external validation to determine if bacteria can be detected in blood and urine

✓ We can detect several types of bacteria in blood and urine reliably



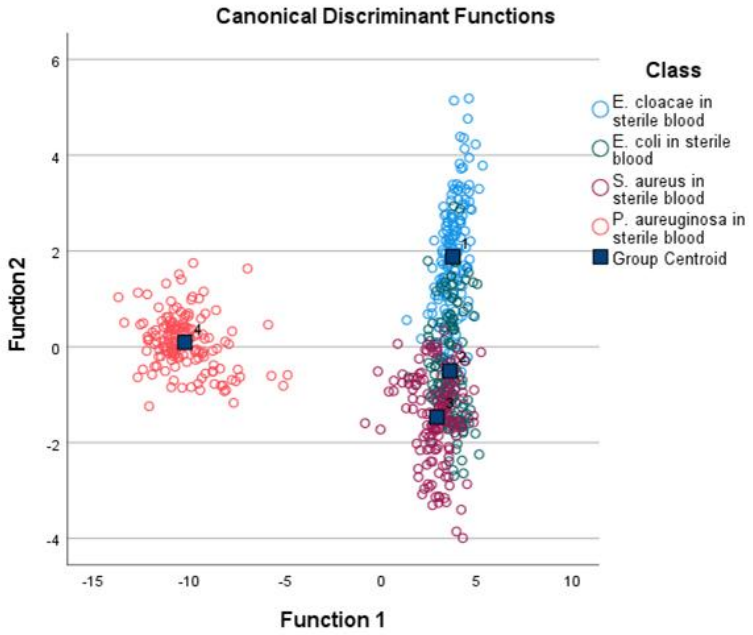
Average Sensitivity: 99.56%
Average Specificity: 100%



Average Sensitivity: 98.90%
Average Specificity: 100%

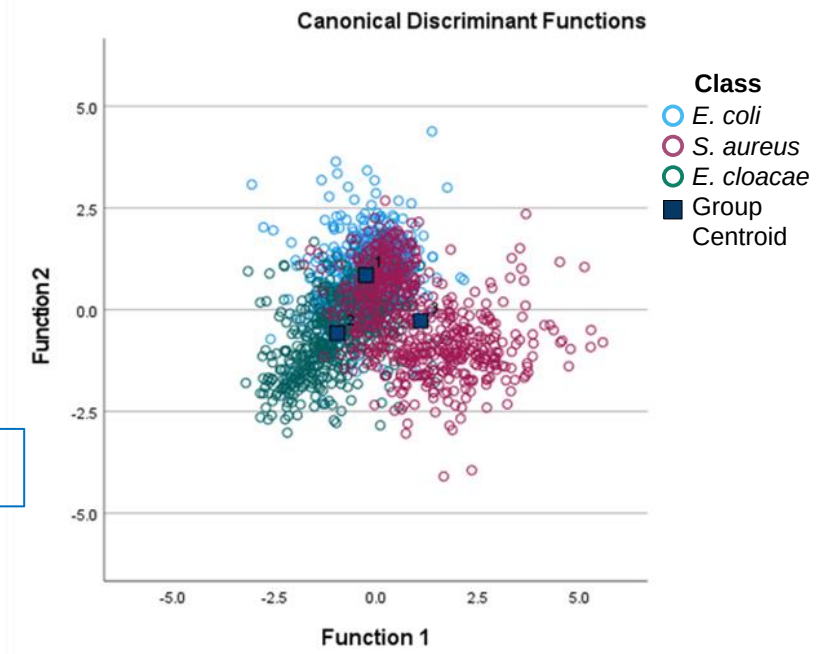
Recent Progress (i): Blood & Urine

Variable down-selection. Ratio-model 2.5: 15 line areas, 92 ratios = 107 variables



4 bacteria in blood

3 bacteria in water



Sample Type (DFA)	Sensitivity	Specificity
Sterile Blood Containing *P. aeruginosa*	98.7 %	100 %
Sterile Blood Containing *S. aureus*	84.0 %	92.9 %
Sterile Blood Containing *E. coli*	74.7 %	93.6 %
Sterile Blood Containing *E. cloacae*	78.0 %	92.0 %

DFA Results			
Sample	Sensitivity (%)	Specificity (%)	Classification Error
E. coli	73.28 %	71.78 %	27.46 %
S. aureus	64.93 %	84.93 %	25.06 %
E. cloacae	60.10 %	92.53 %	23.68 %

Recent Progress (ii): ANN. First alteration

- We implemented a feed forward, one-hidden layer ANN in Python.
- First optimized the # of epochs for a certain number of nodes.
- Used an algorithm that calculates sensitivity/specificity as a function of nodes and patience (100-140, 40).

DFA on RM2.5

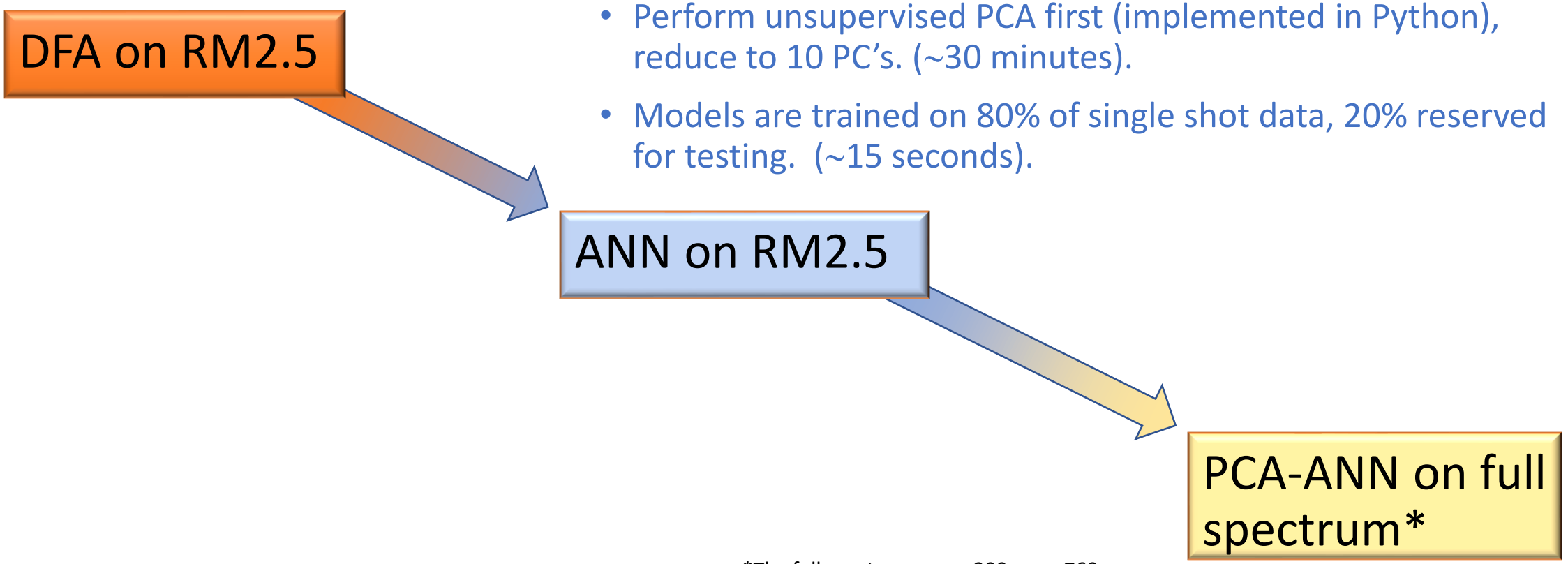
ANN on RM2.5

Sample Type (ANN)	Sensitivity	Specificity
Sterile Blood Containing *P. aeruginosa*	97.2 %	100 %
Sterile Blood Containing *S. aureus*	100 %	100 %
Sterile Blood Containing *E. coli*	100 %	100 %
Sterile Blood Containing *E. cloacae*	100 %	98.9 %
Sample Type (DFA)	**Sensitivity**	**Specificity**
Sterile Blood Containing *P. aeruginosa*	98.7 %	100 %
Sterile Blood Containing *S. aureus*	84.0 %	92.9 %
Sterile Blood Containing *E. coli*	74.7 %	93.6 %
Sterile Blood Containing *E. cloacae*	78.0 %	92.0 %

ANN Results			
Sample	**Sensitivity (%)**	**Specificity (%)**	**Classification Error**
E. coli	98.0 %	97.7 %	2.13 %
S. aureus	93.3 %	97.2 %	4.28 %
E. cloacae	91.2 %	96.1 %	6.33 %
DFA Results			
Sample	**Sensitivity (%)**	**Specificity (%)**	**Classification Error**
E. coli	73.28 %	71.78 %	27.46 %
S. aureus	64.93 %	84.93 %	25.06 %
E. cloacae	60.10 %	92.53 %	23.68 %

Recent Progress (ii): ANN. Second alteration

- 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. (~30 minutes).
- Models are trained on 80% of single shot data, 20% reserved for testing. (~15 seconds).



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

Recent Progress (ii): ANN. Results.

- When using the 80:20 single shot data, performance is always essentially 100%.
- But this is an incorrect test. External validation is the only accurate test. ⇒ Testing unique specimens/patients.

3 bacteria in water

		actual		
		E. coli	*Staph*	*Myco*
predicted	*E. coli*	436	10	0
	Staph	12	387	1
	Myco	1	7	418

	E. coli	*Staph*	*Myco*
sens	0.971047	0.957921	0.997613
spec	0.987849	0.985023	0.990621

Escherichia coli

E. COLI	Predicted			
Sample	e. coli	staph	myco	Sensitivity
80321	30	0	0	100
102521	30	0	0	100
22322	28	2	0	93.33333
022322-1	24	6	0	80
32122	30	0	0	100
032122-2	30	0	0	100
41122	30	0	0	100
041122-2	30	0	0	100
041122-3	30	0	0	100
041122-4	30	0	0	100
041122-5	30	0	0	100
041122-6	30	0	0	100
041122-7	30	0	0	100
41322	28	2	0	93.33333
041322-2	26	2	1	89.65517
Sum	436	12	1	97.10468

Staphylococcus aureus

STAPH	Predicted			
Sample	e. coli	staph	myco	Sensitivity
41422	7	23	0	76.66667
81221	0	30	0	100
82421	0	15	0	100
22322	0	30	0	100
022322-2	0	25	5	83.33333
022322-3	0	30	0	100
022322-4	0	30	0	100
22422	0	30	0	100
022422-2	3	27	0	90
022422-4	0	28	1	96.55172
22522	0	30	0	100
022522-2	0	29	1	96.66667
022522-3	0	30	0	100
031822-2	0	30	0	100
Sum	10	387	7	95.79208

Mycobacterium smegmatis

MYCO	Predicted			
Sample	e. coli	staph	myco	Sensitivity
081522-1	0	0	30	100
081522-2	0	0	30	100
081522-3	0	0	30	100
081522-4	0	0	30	100
081522-5	0	0	30	100
081522-6	0	0	30	100
081522-7	0	0	30	100
081522-8	0	0	30	100
081522-9	0	1	29	96.66667
081522-10	0	0	30	100
081622-1	0	0	30	100
081622-2	0	0	30	100
081622-3	0	0	29	100
081622-4	0	0	30	100
Sum	0	1	418	99.76134

Data set is 42,000 x 1272

Recent Progress (ii): ANN. Results.

- When using the 80:20 single shot data, performance is always essentially 100%.
- But this is an incorrect test. External validation is the only accurate test. ⇒ Testing unique specimens/patients.

4 bacteria in blood

		actual			
		Staph	*E. coli*	*Entero*	*Pseudo*
predicted	*Staph*	98	17	9	8
	E. coli	23	121	1	1
	Entero	29	0	139	0
	Pseudo	0	12	1	111

	Staph	*E. coli*	*Entero*	*Pseudo*
sens	0.653333	0.806667	0.926667	0.925
spec	0.919048	0.940476	0.930952	0.971111

Staphylococcus aureus

STAPH	Predicted				
	staph	**e. coli**	**entero**	**pseudo**	Sensitivity
031122-1	30	0	0	0	1
031122-2	1	0	29	0	0.0333333
041422-1	30	0	0	0	1
041422-2	30	0	0	0	1
041422-3	7	23	0	0	0.2333333
Sum	98	23	29	0	0.6533333

Escherichia coli

E. COLI	Predicted				
	staph	**e. coli**	**entero**	**pseudo**	Sensitivity
032522-1	0	30	0	0	1
032522-2	16	14	0	0	0.4666667
040122-1	0	30	0	0	1
040122-2	1	17	0	12	0.5666667
040122-3	0	30	0	0	1
Sum	17	121	0	12	0.8066667

ENTERO	Predicted				
	staph	**e. coli**	**entero**	**pseudo**	Sensitivity
032922-1	0	0	30	0	1
032922-2	9	0	21	0	0.7
032922-3	0	0	29	1	0.9666667
032922-4	0	0	30	0	1
032922-6	0	1	29	0	0.9666667
Sum	9	1	139	1	0.9266667

Enterobacter cloacae

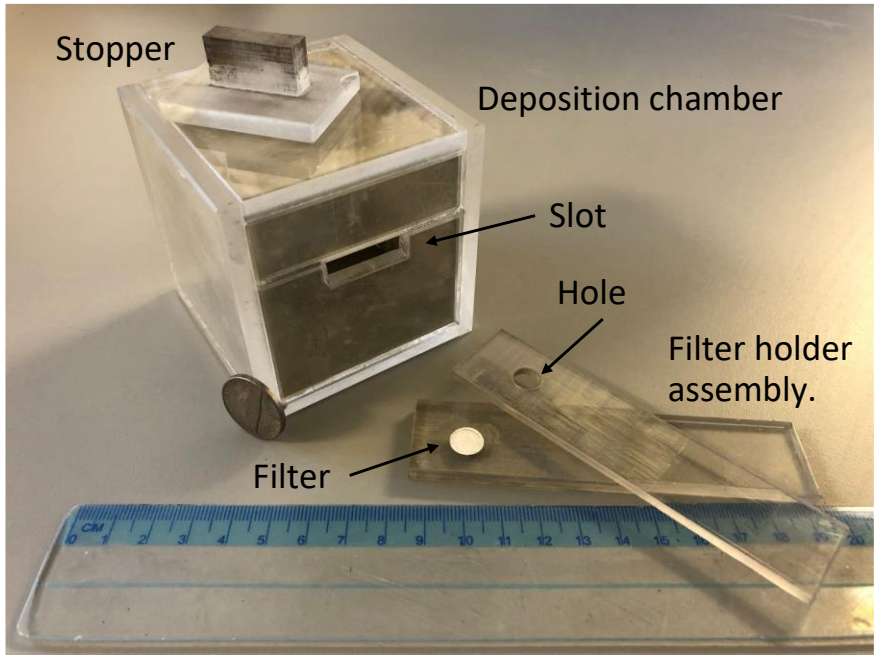
PSE					
					vity
071					667
071					333
071					1
071					1
Sum					925

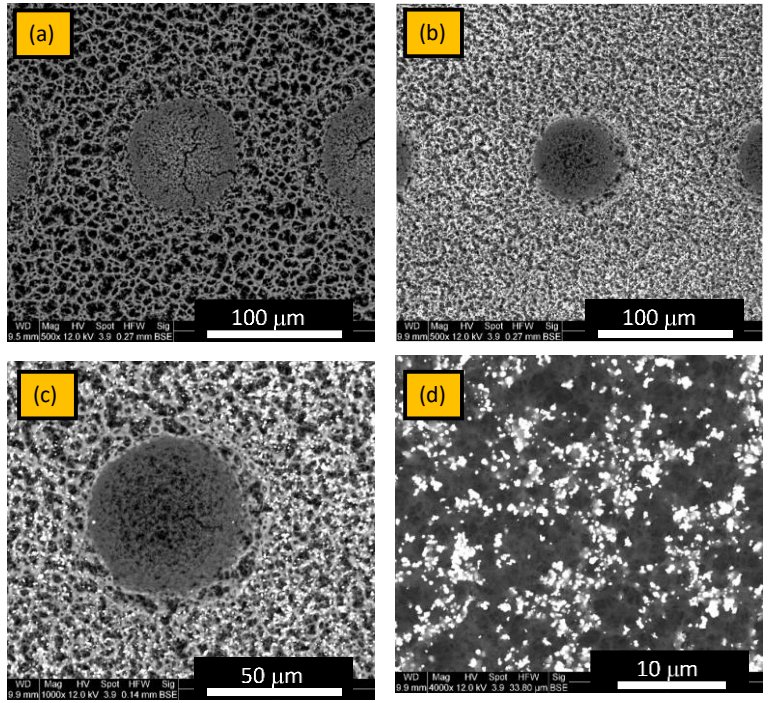
Pseudomonas aeruginosa

Our data tends to "fail by filter."

We are revising our mounting method.

Recent Progress (iii): Using silver.





- 0.5 - 1.0 µm spheres
- 99.9% Ag
- 3.3 ng/shot

Table I. The average enhancement ratio for all emission lines for a given element in a LIBS spectrum of bacterial cells ablated in the presence of silver microparticles.

Bacteria Species	Elements				
	C	P	Mg	Ca	Na
E. coli	1.3	4.6	3.9	5.4	3.9
M. smegmatis	1.2	1.7	2.7	8.4	6.7
E. cloacae	1.2	4.4	6.9	2.2	1.3

Special Issue: Laser Induced Breakdown Spectroscopy

Spectroscopy

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Silver Microparticle-Enhanced Laser-Induced Breakdown Spectroscopy

Applied Spectroscopy
2022, Vol. 76(8) 905–916

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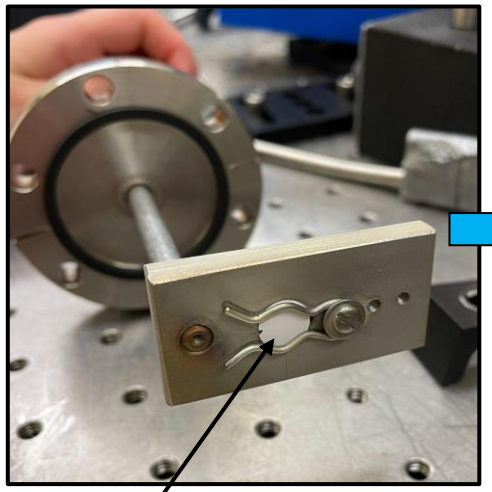
University of Windsor

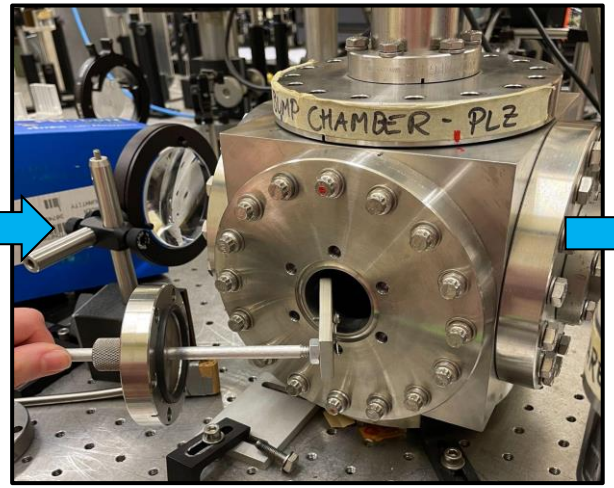
Pulsed Laser Sputtering

A 1064 nm pulsed laser (60 mJ/pulse) is focused onto a rotating silver target inside a vacuum chamber.

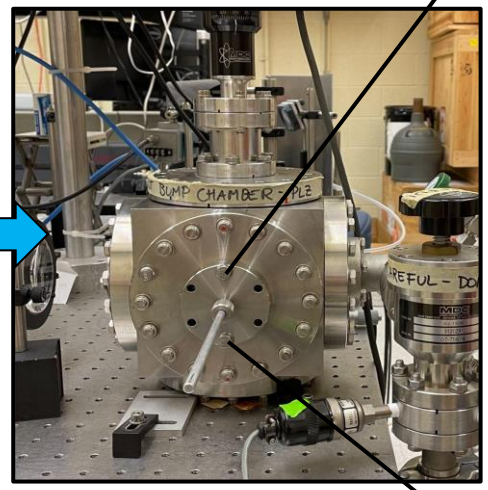
Experimental setup:



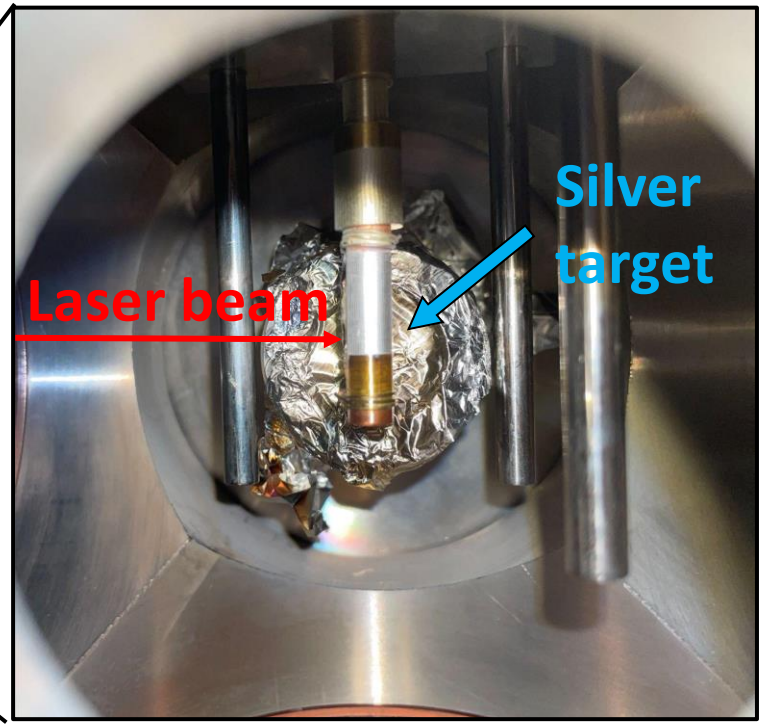
Filter on custom **aluminum filter holder**



Filter holder being inserted into **10 mTorr evacuated chamber**



Closed vacuum chamber apparatus



Inside the chamber

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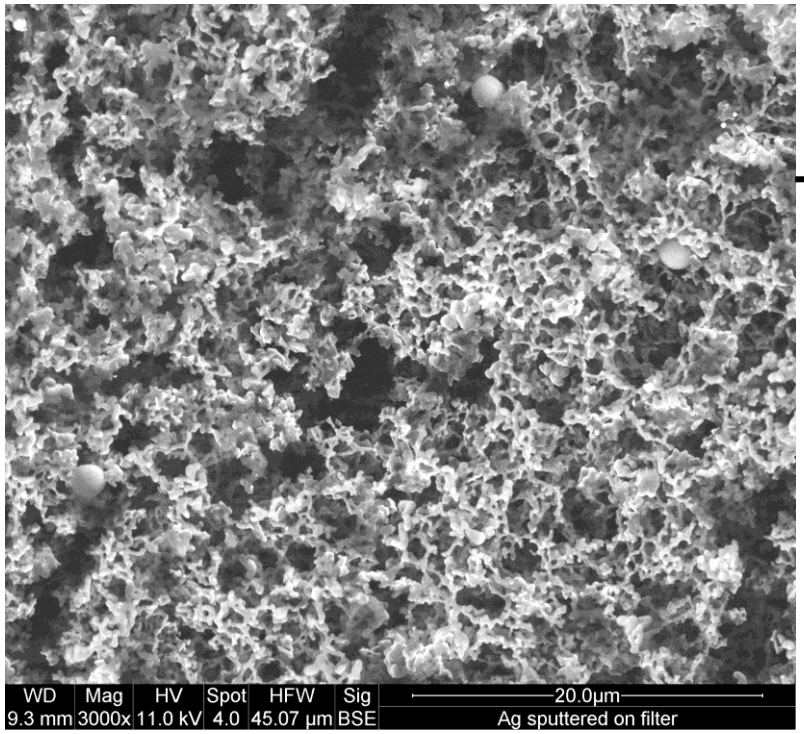
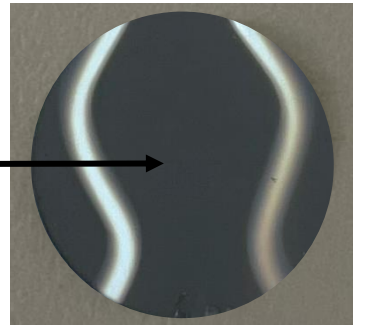
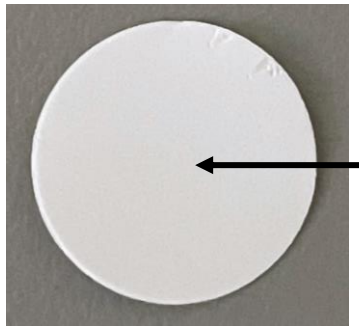
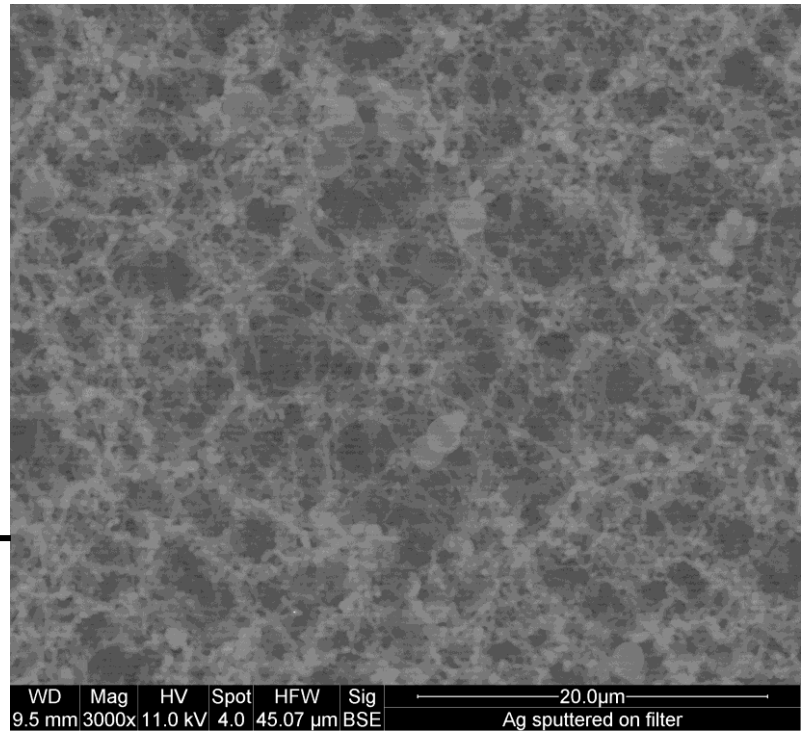
Results: Silver Film Analyzed with Scanning Electron Microscope (SEM)









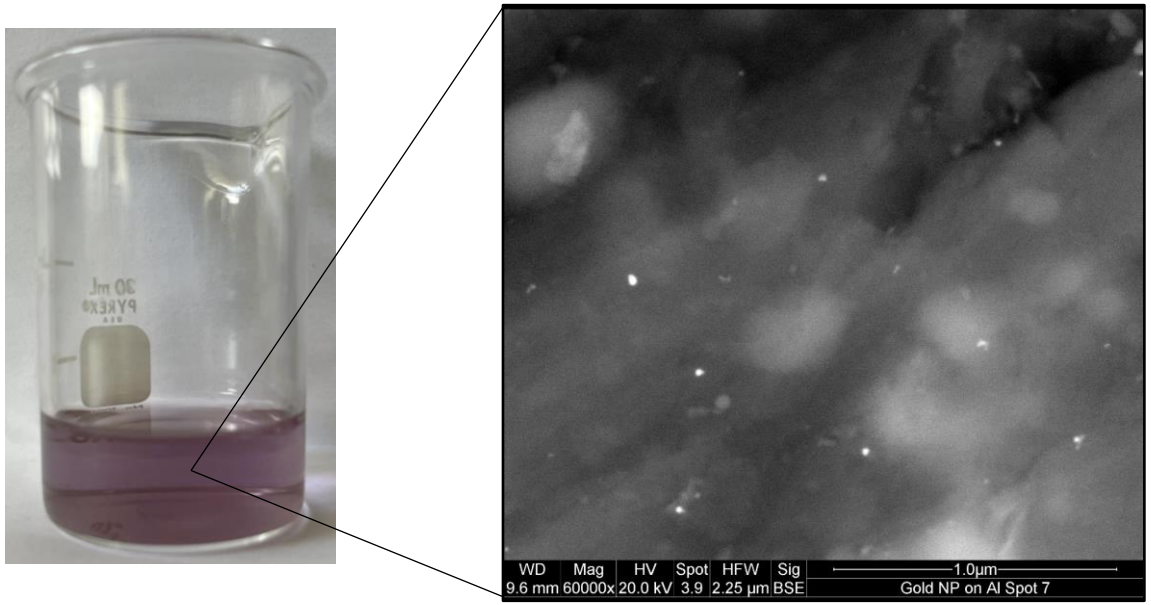
Filter with 20 min Ag

Blank Filter

The structure of the filter is unchanged but it is clear that silver has coated the filter.

Future Work

- Use nano-particle enhanced LIBS (NELIBS) to increase the emission intensity of our bacteria
- We have successfully produced a gold nanoparticle suspension using pulsed laser ablation in liquid (PLAL)



Au Nanoparticle suspension

Next Steps:

Investigate (i) laser pulse energy, (ii) volume of liquid, (iii) sputtering time to determine effect on size and spacing of the NP's.

Deposit NP's on filter or suspend with bacteria to investigate NELIBS.

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