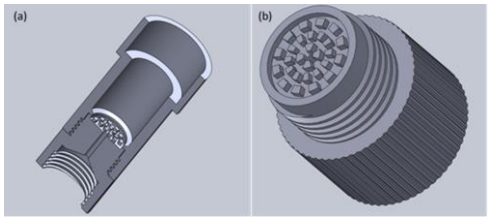
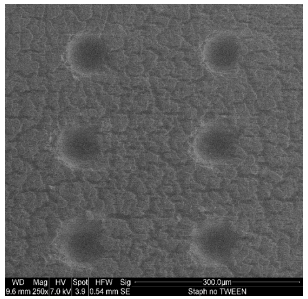
FACSS PRESENTS
SCIX2019
THE GREAT SCIENTIFIC EXCHANGE
OCTOBER 13 – 18 | PALM SPRINGS, CA

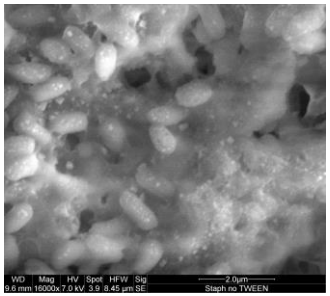
Bacterial Limit of Detection Reduction Utilizing a Novel Sample Preparation Protocol

Steven J. Rehse, Jeremy Marvin, Alexandra E. Paulick, Emma Blanchette, Sydney Sleiman

Department of Physics, University of Windsor, Windsor, Ontario, Canada





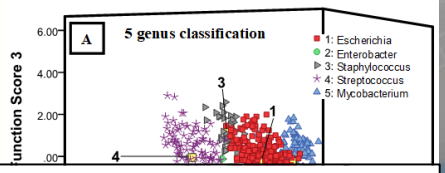


University of Windsor

2005-2011

How unique is "unique"?

✓ We can identify a bacterial species, certainly its genus, with high sensitivity and



8 publications in Spectroscopy

Spectrochimica Acta Part B 154 (2019) 50–69

Contents lists available at ScienceDirect

Spectrochimica Acta Part B

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

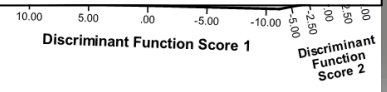
Invited Review

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

DFA: *Sensitivity*: 91.37 ± 16.39 % *Specificity*: 97.46 ± 9.35 %
PLSDA: *Sensitivity*: 93.13 ± 10.25 % *Specificity*: 90.60 ± 21.33 %

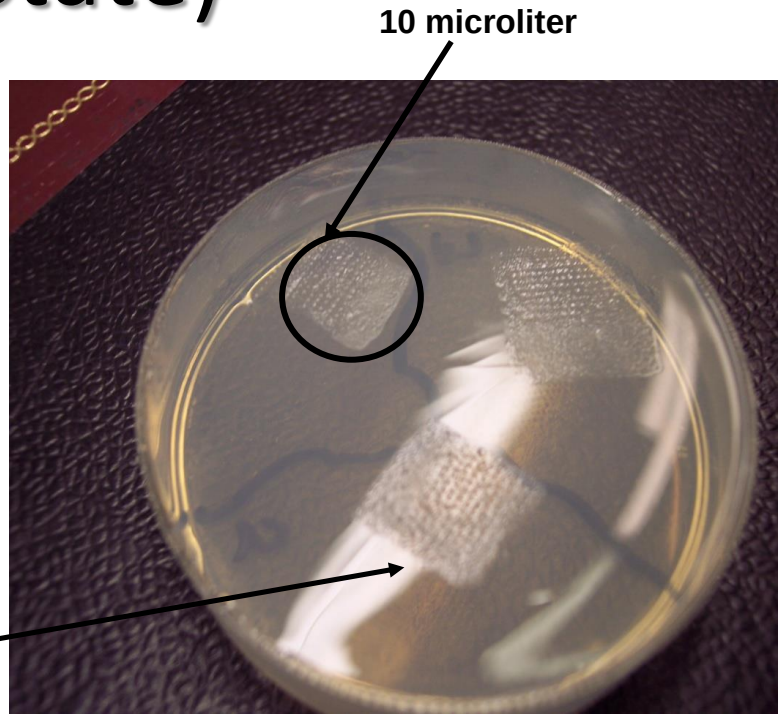


2011-2019

Can we develop this to be a convenient and easy-to-use (for clinicians) test?

University of Windsor

Early days (at Wayne State)



Nutrient-free bacto-agar

Advantages:

✓ Background free mounting substrate

Disadvantages:

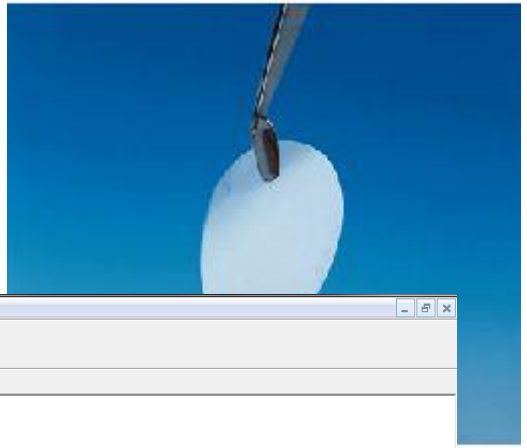
X Not really flat

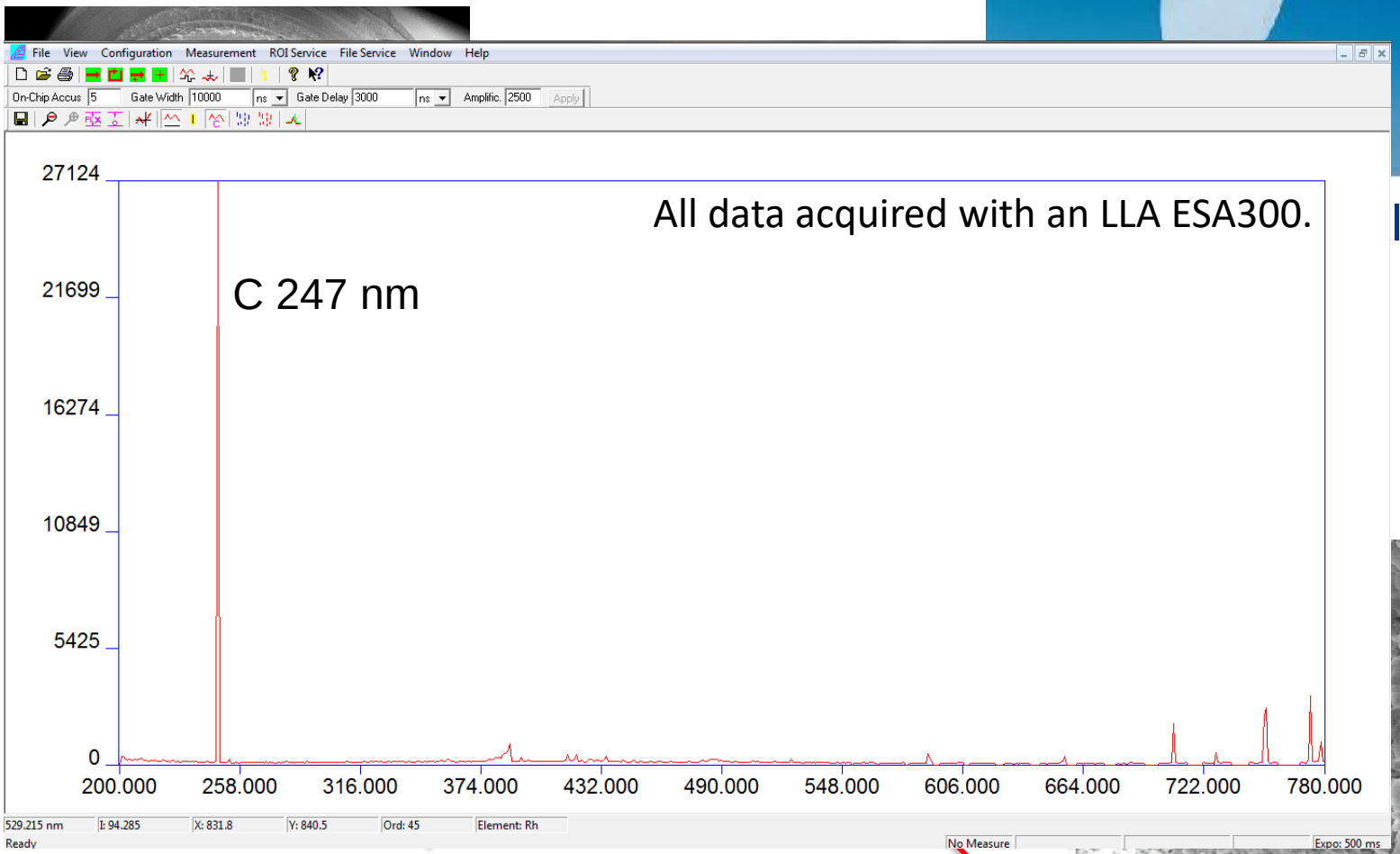
X Degrades

X Hard to make

X Watery

Moving to Filters (Early Days at Windsor)

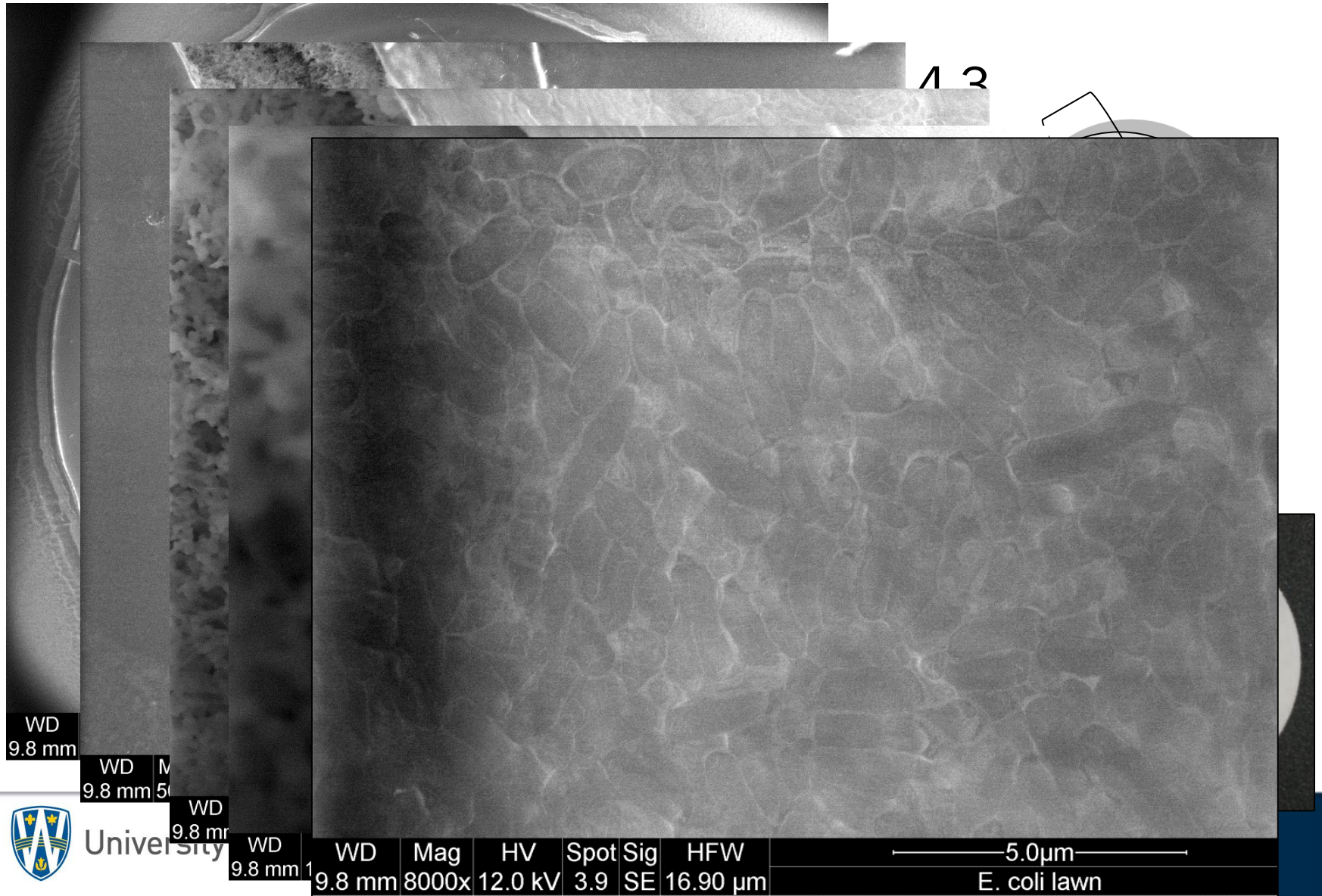




lters

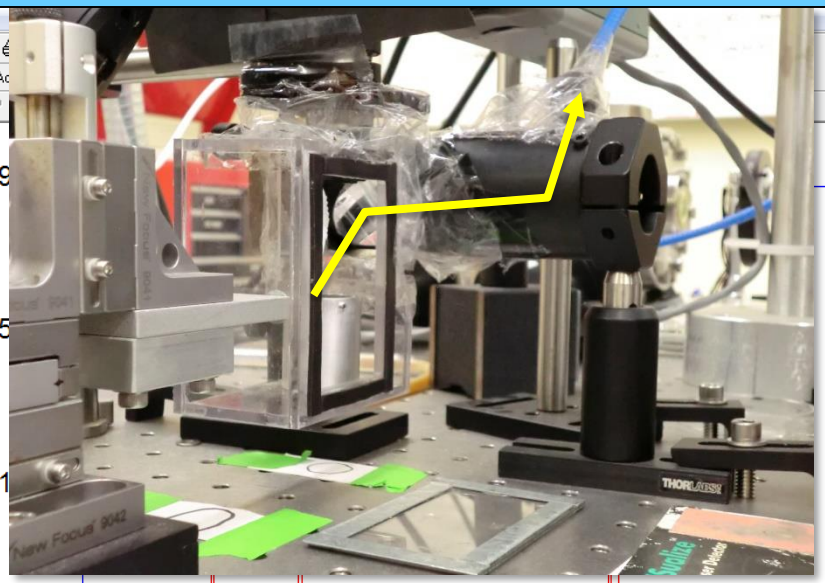
How to get bacteria onto filters?

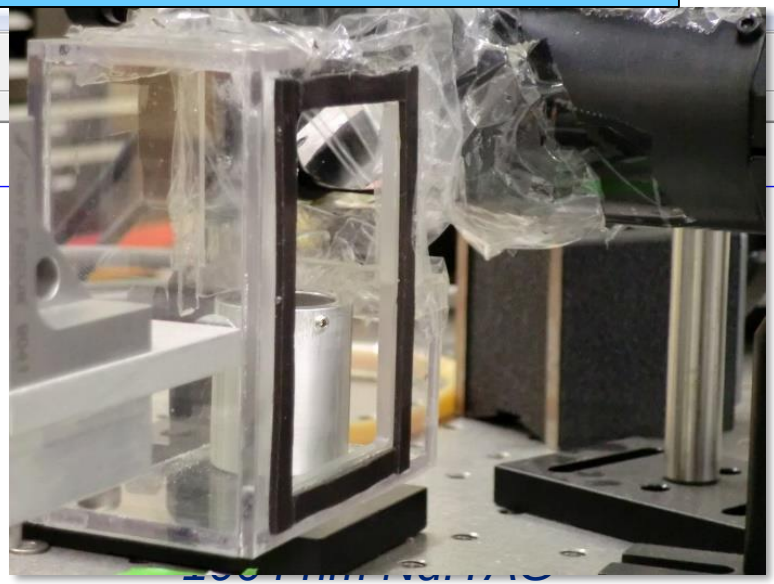
How to get bacteria onto filters?



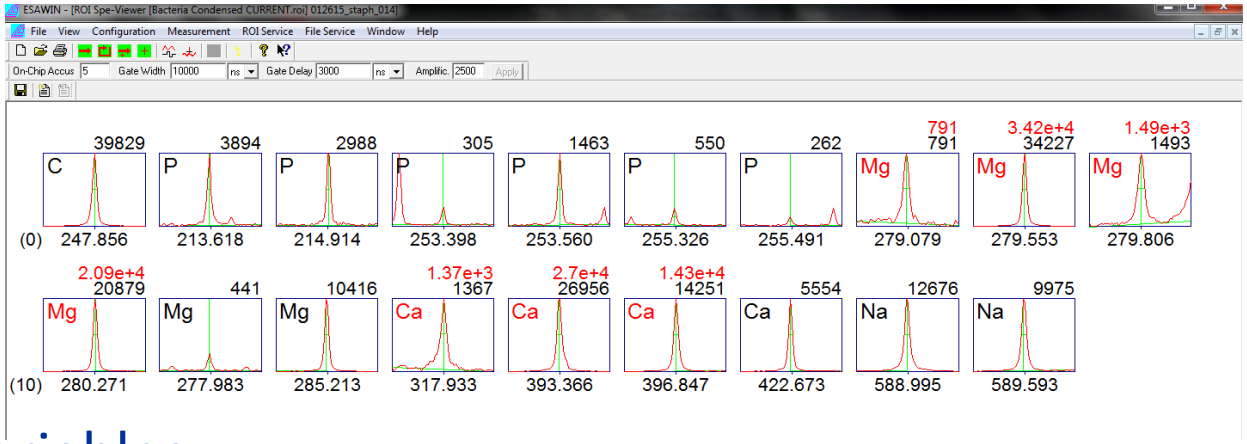
Typical bacterial LIBS spectrum

Currently: using matched parabolic reflectors into fiber for UV



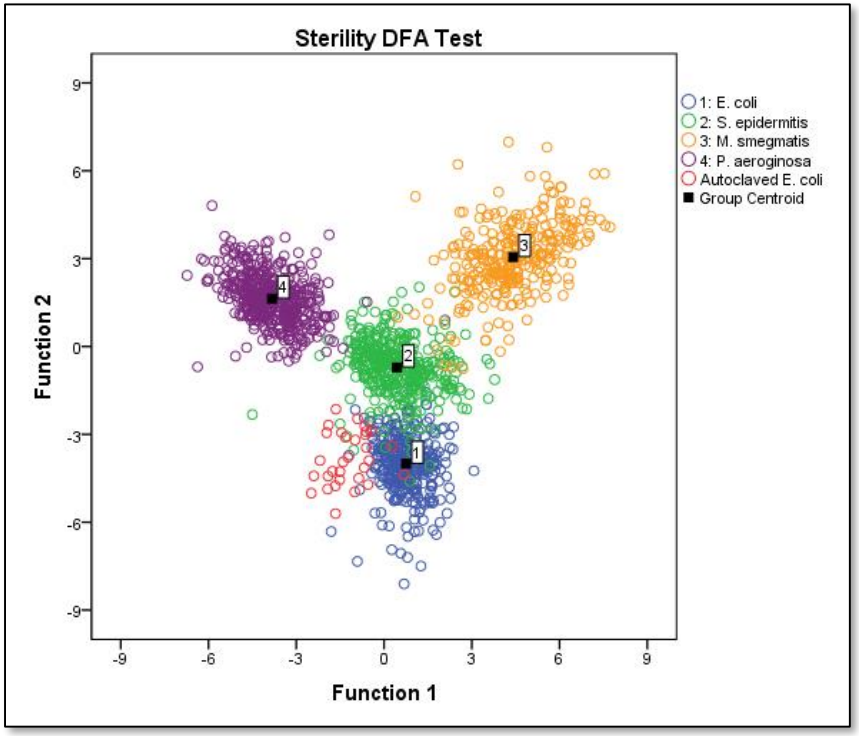


Data analysis with variable down selection



- 164 independent variables
  - 19 line intensities (all divided by sum) of C, P, Mg, Ca, Na
  - 145 ratios of intensities

Performance on filters



DFA Classification Grouped by Species

Escherichia	TRUE	FALSE	*Staphylococcus*	TRUE	FALSE
Positive	98.28%	0.77%	Positive	97.75%	1.44%
Negative	99.23%	1.72%	Negative	98.56%	2.25%
Mycobacterium	TRUE	FALSE	***Pseudomonas***	TRUE	FALSE
Positive	95.36%	0.33%	Positive	99.57%	0.22%
Negative	99.67%	4.64%	Negative	99.78%	0.43%

Sensitivity: 98 ± 2% Specificity: 99 ± 1%

PLS-DA Classification Grouped by Species

Escherichia	TRUE	FALSE	*Staphylococcus*	TRUE	FALSE
Positive	96.55%	1.12%	Positive	96.75%	1.53%
Negative	98.88%	3.45%	Negative	98.47%	3.25%
Mycobacterium	TRUE	FALSE	***Pseudomonas***	TRUE	FALSE
Positive	97.02%	0.41%	Positive	98.92%	0.33%
Negative	99.59%	2.98%	Negative	99.67%	1.08%

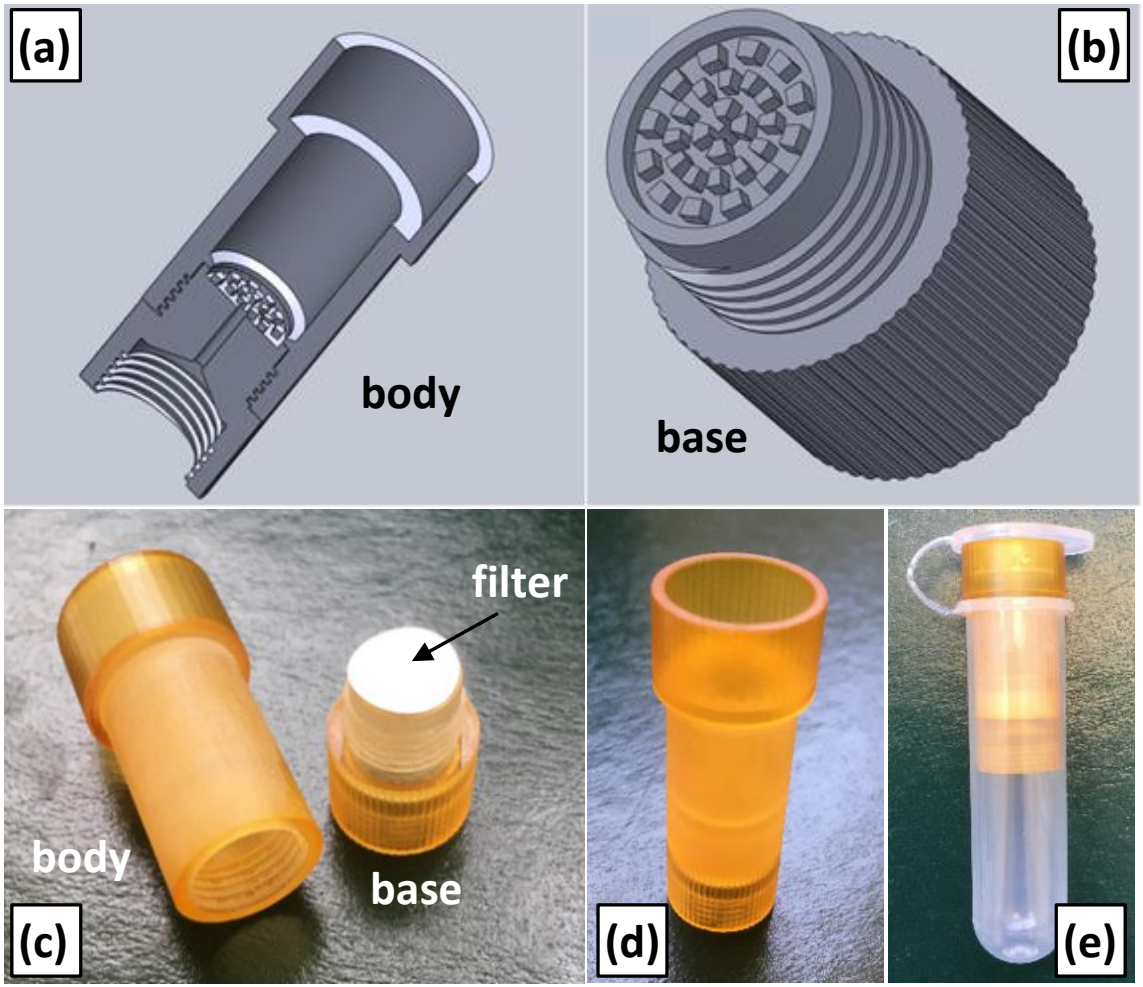
Sensitivity: 97 ± 3% Specificity: 99 ± 2%

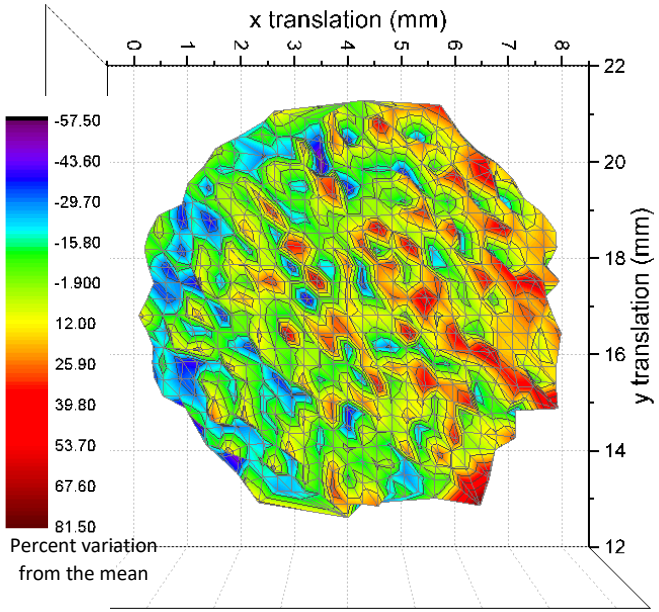
All external validation results

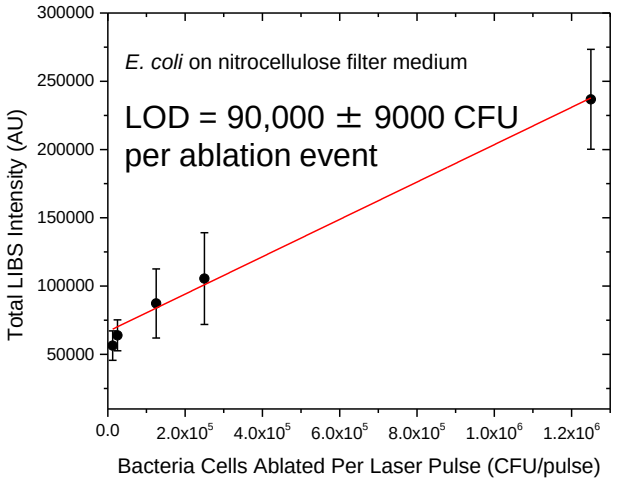
	DFA (by filter)	**DFA (by species)** above	**PLSDA (by species)** above
Sensitivity	0.93±0.07	0.98±0.02	0.97±0.03
Specificity	0.98±0.03	0.99±0.01	0.99±0.02

"by filter" means approximately 30 groups in DFA, no relationships between groups assumed

Using filters, a better way: the centrifuge insert

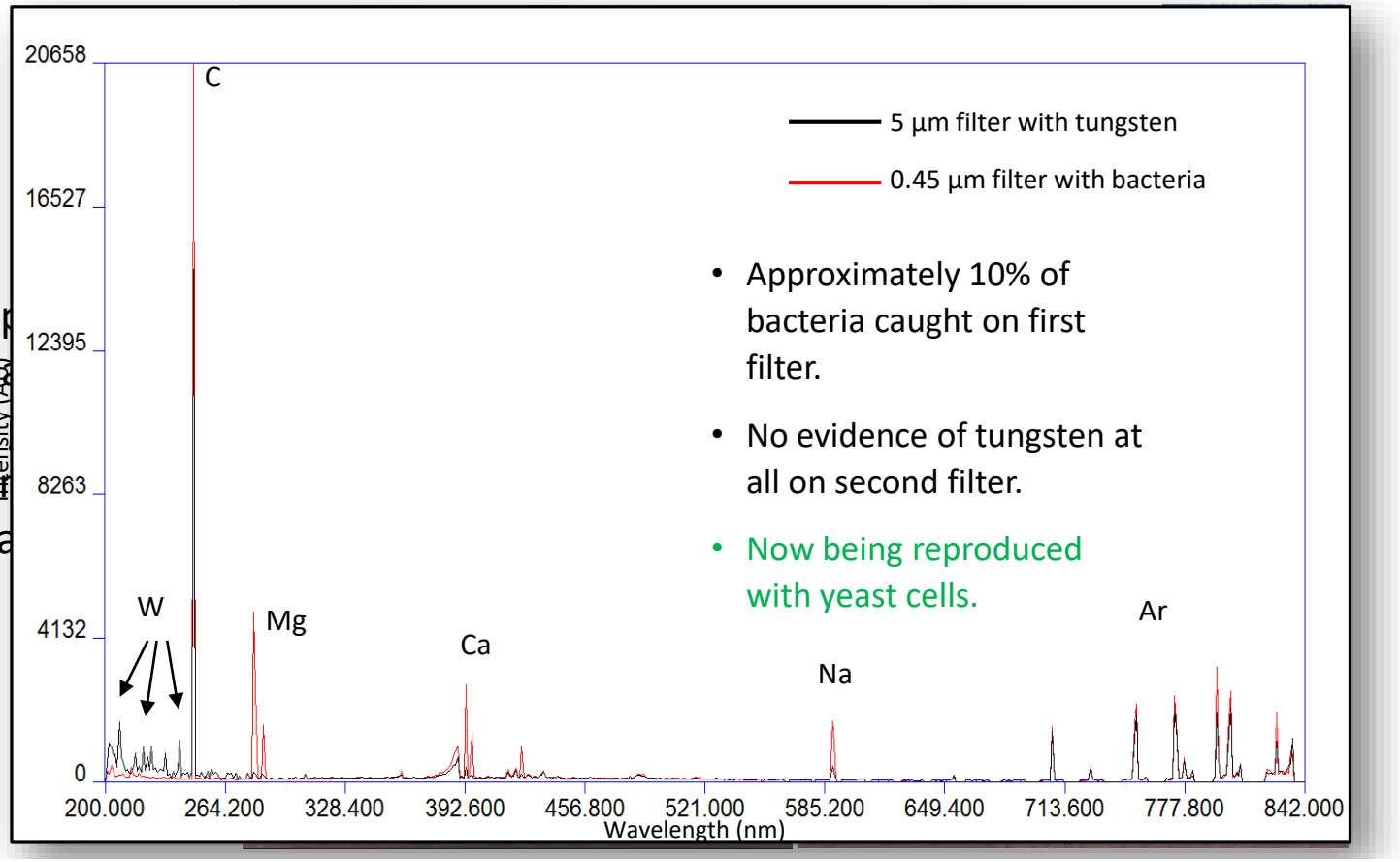






The centrifuge insert for cell sorting

Tungsten p
average p
size of 12
used to s
contamina



- Tungsten powder was chosen for its inertness, cost and availability, safety of use, ease of preparation, biologically relevant size, and elemental purity.
- Tungsten powder was added to a suspension of *E. coli*, vortexed, and ~ 0.1 mL was pipetted into the top of the insert prior to centrifugation.

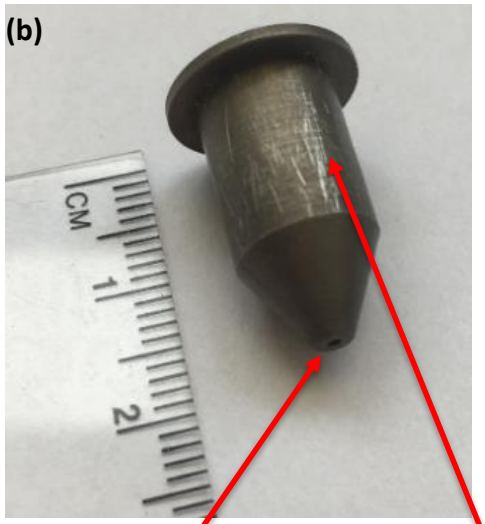
Using filters, a better way: the centrifuge insert

Remember?

Using filters, an even better way: the centrifuge cone

19 mm long Al cone

Centrifuge tube cap presses cone into filter

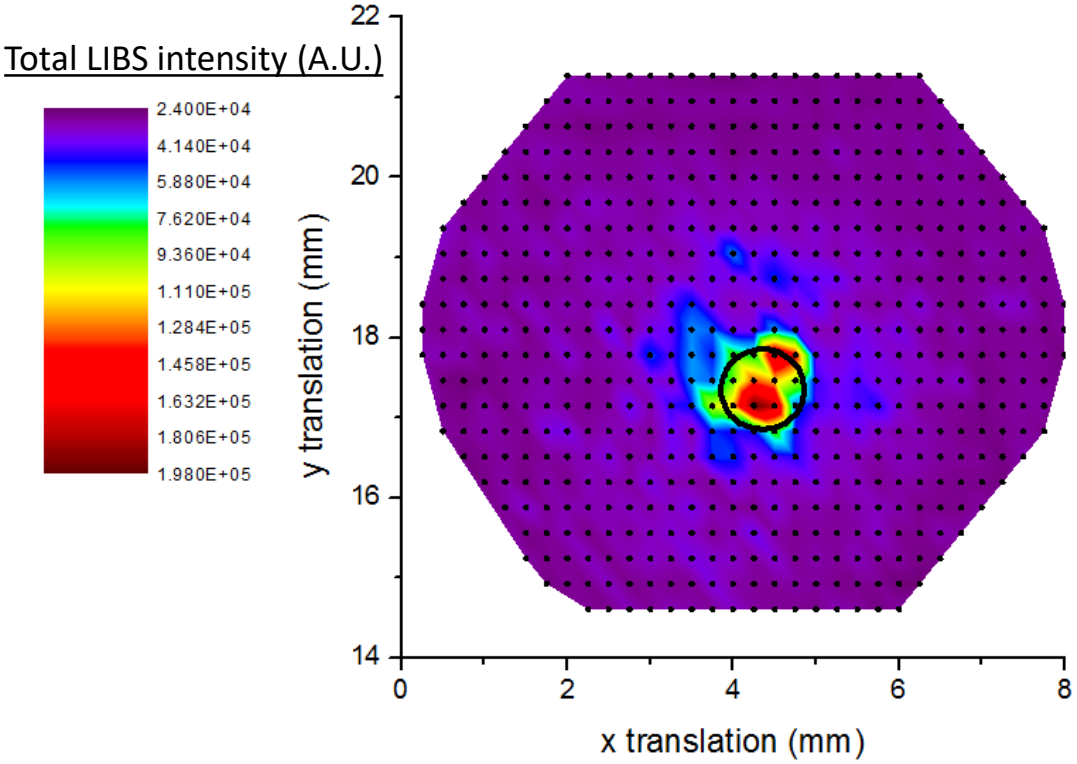


1 mm hole at apex

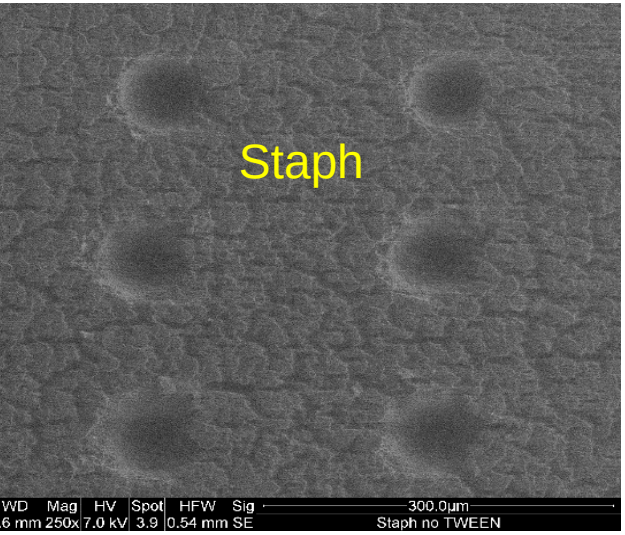
Holds 1 mL of fluid

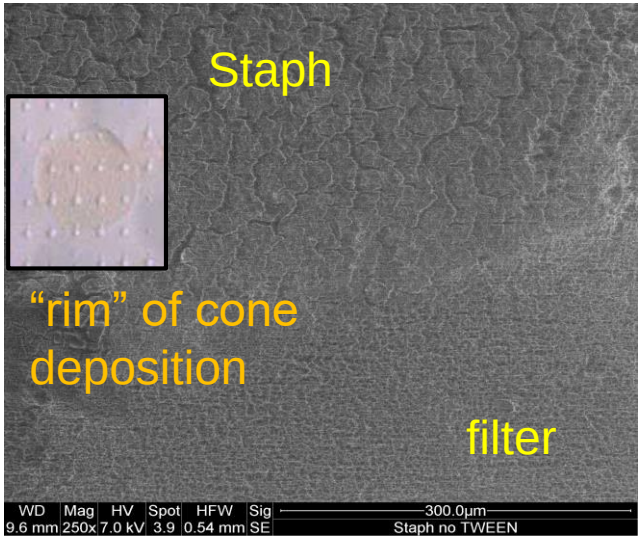
Cone vertex press fit into filter

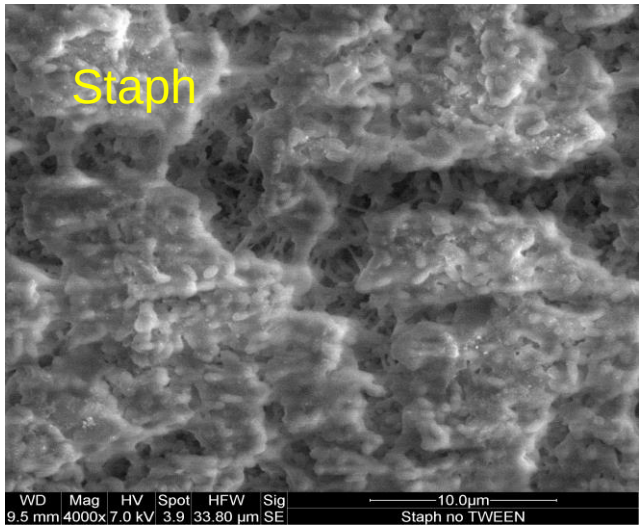
Using filters, an even better way: the centrifuge cone

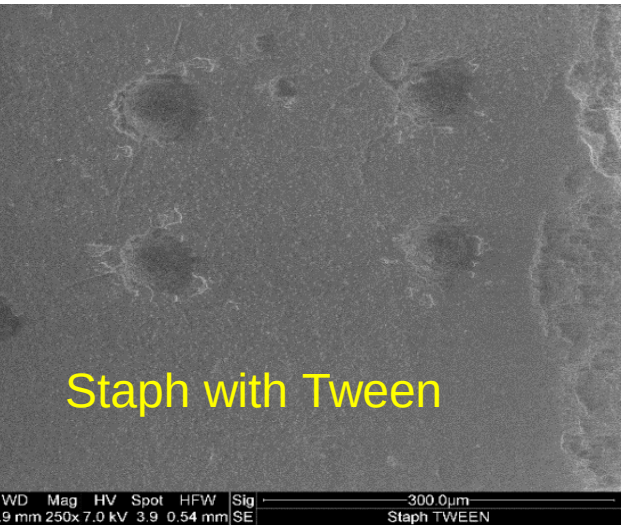


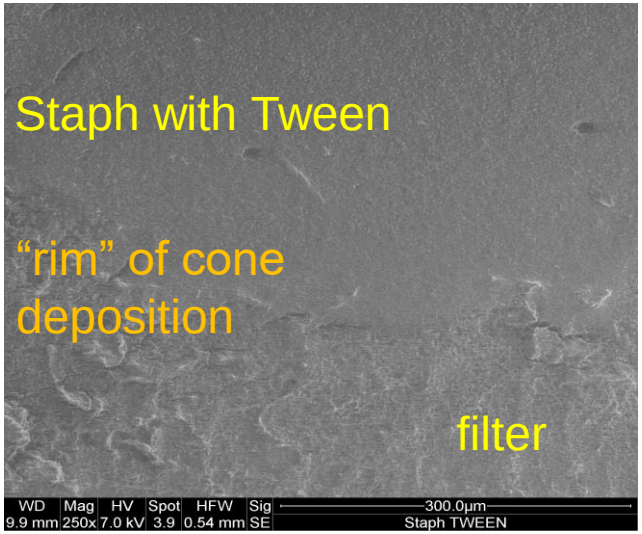
SEM micrographs

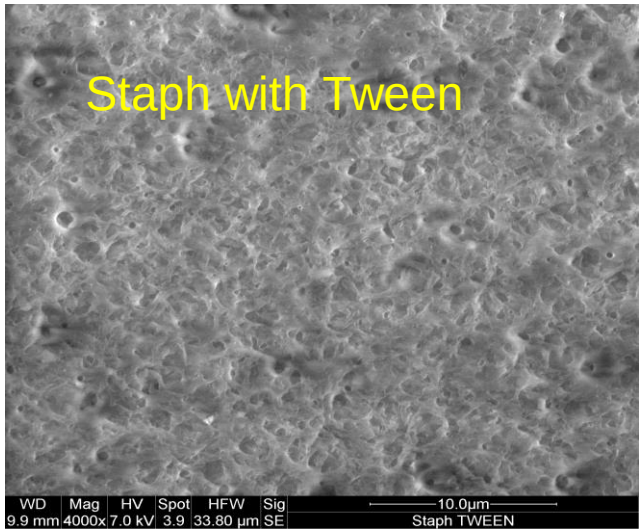




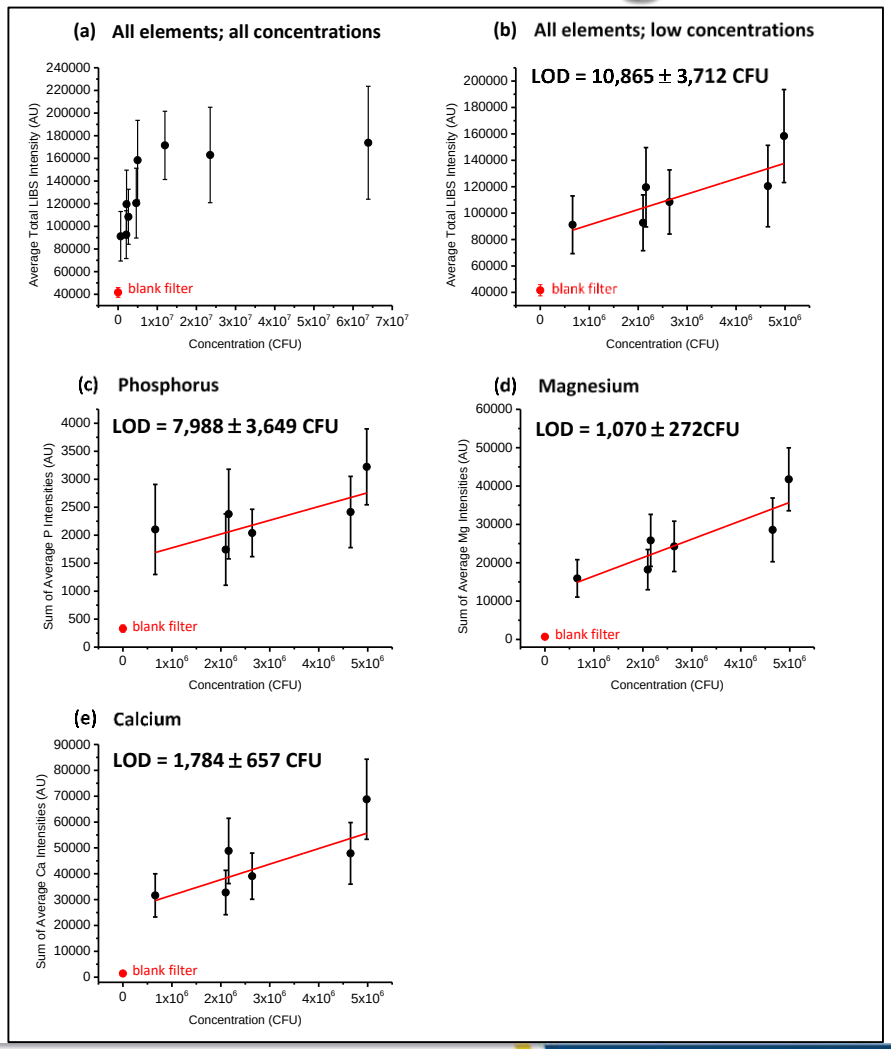








Using filters, an even better way: the centrifuge cone



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:

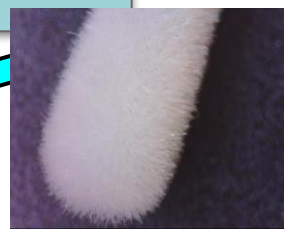
1,070 ± 272CFU for magnesium
1,784 ± 657 CFU for calcium.

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

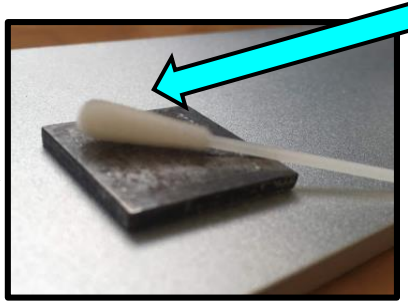
Collecting on swabs

So little bacterial sample is needed that our specimens are all collected from clinical flocked swabs.

The swab was tested for traces of contamination and did not contribute to background emission.

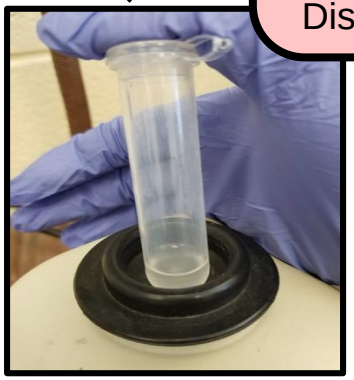


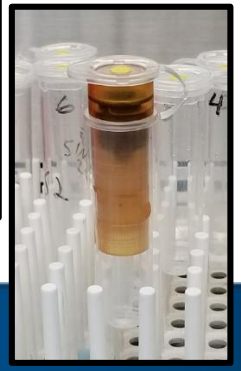
Deposit 100 µL of bacteria suspended in DI water onto metal plate and heat for 2 min and 20 s.



Deposit 10 µL water onto swab tip and swab metal plate.

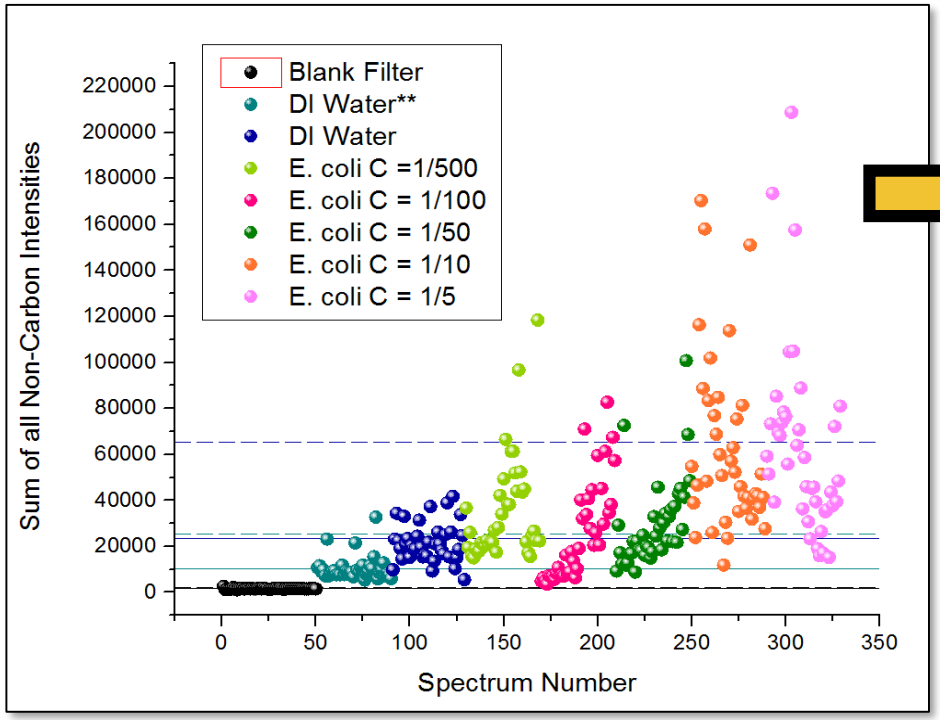
Place swab in centrifuge tube with 1 mL of water and vortex tube for 15 s. Discard swab.

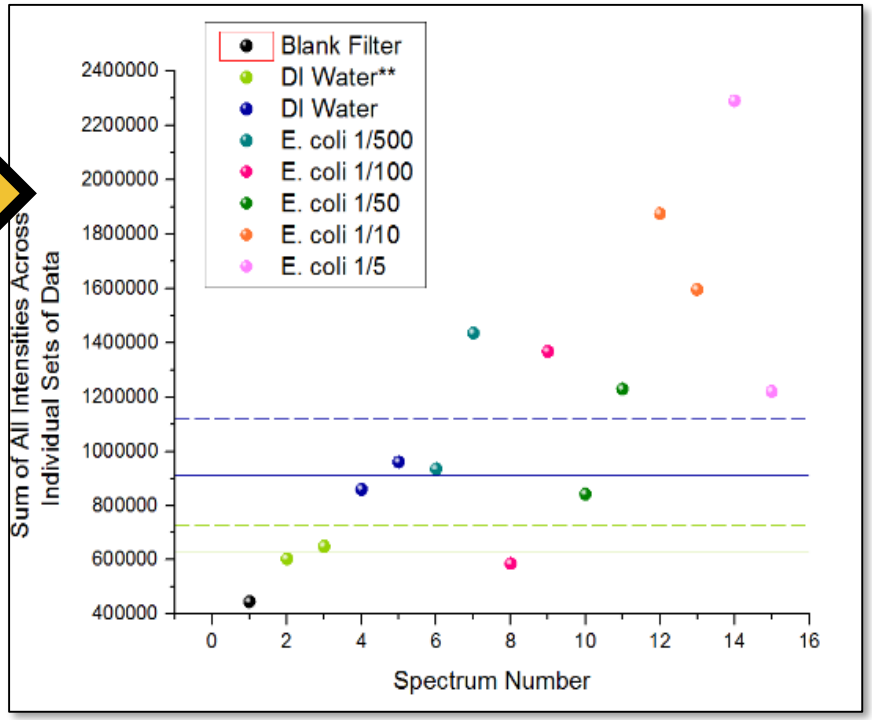




Deposit the 1 mL of sample from vortexed tube into cone, place insert into clean centrifuge tube. Centrifuge insert at 5000 rpm, 2500 g's for 5 min

Collecting on swabs



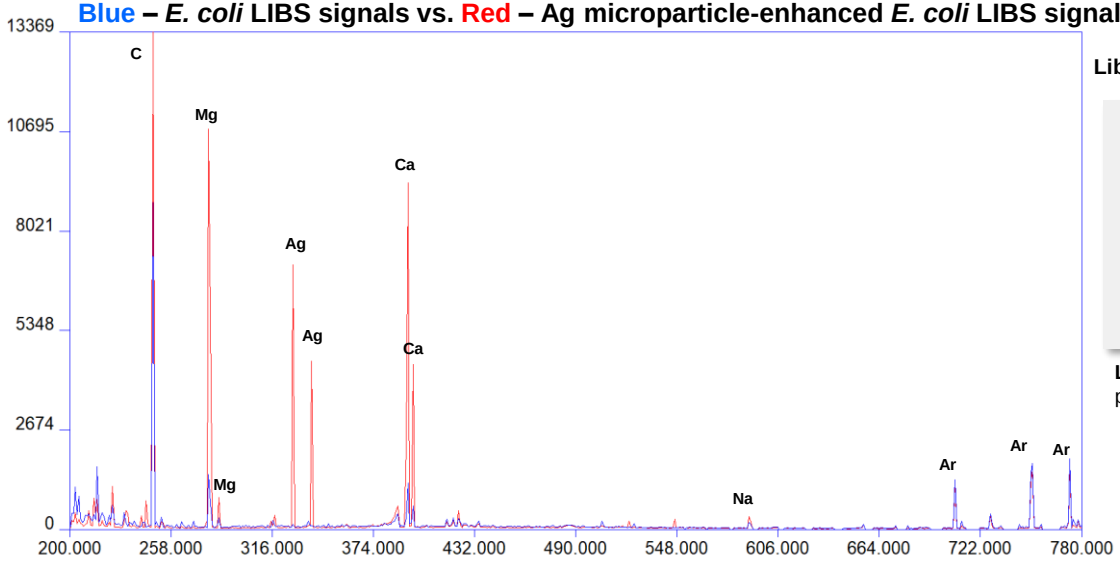


Two separate filters, 20 shots / filter (all single shot spectra).

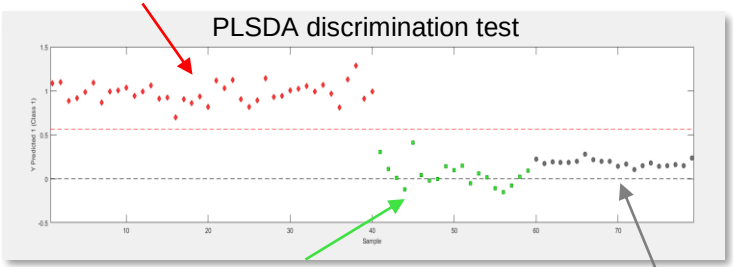
Summing intensities of spectral lines.

Ag microparticle-enhanced LIBS

Blue – *E. coli* LIBS signals vs. Red – Ag microparticle-enhanced *E. coli* LIBS signals



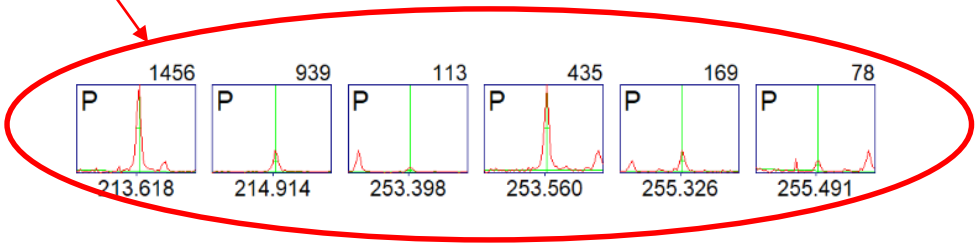
Library: *E. coli* K12 on two filters



Library: *E. coli* K12 on filter with Ag micro particles

Test: *E. coli* K12 on filter with Ag micro particles

	C	P	P	P	P	P	P	Mg	Mg	Mg	Mg	Mg	Mg	Ca	Ca	Ca	Ca	Na	Na
ecoli																			
Average	17398.75	262.1579	129.6667	45.6	85.29412	44	40.2	73.26316	2657.85	150.1176	1386.65	31.63636	477.8	107.3889	1847.95	905.2778	336.45	294.65	208.35
Ag filter																			
Average	22027.7	1489.9	967.4	116.75	454.45	183.6	97.38889	209.85	10976.2	403.85	6445.4	110.8	2729	545.5	10609.75	5267.6	1632.2	1182.95	799.6
Ratio ecoli	1.266051	5.683216	7.460668	2.560307	5.328034	4.172727	2.422609	2.864332	4.129729	2.690223	4.648181	3.502299	5.711595	5.079669	5.741362	5.818766	4.851241	4.014763	3.837773
Ratio Myco	1.052175	1.283082	1.034126	1.200637	1.030807	0.690789	1.173228	0.758315	0.860698	0.66146	0.905237	1.02381	0.654355	**2.711003**	1.885389	1.880476	1.134499	**2.238825**	**2.010381**
Ratio Pseudo	1.325782	1.15621	1.556767	1.087019	0.821082	0.796933	1.205788	**3.326932**	**15.23731**	**5.46253**	**13.47256**	0.830553	**3.034346**	**32.88412**	**35.45526**	**33.85862**	**6.919711**	1.096589	0.941077



Methodology: 0.5 - 1 micron silver powder was transferred via swab and spread uniformly on nitrocellulose filter which was then placed facedown on a second filter and pressed lightly to transfer trace amounts of the powder.

The people who did the work…

NSERC Discovery Grant

Natural Sciences and Engineering Research Council of Canada

Conseil de recherches en sciences naturelles et en génie du Canada

CFI-LOF grant

INNOVATION.CA

CANADA FOUNDATION FOR INNOVATION | FONDATION CANADIENNE POUR L'INNOVATION

University of Windsor

University of Windsor

Thank you!

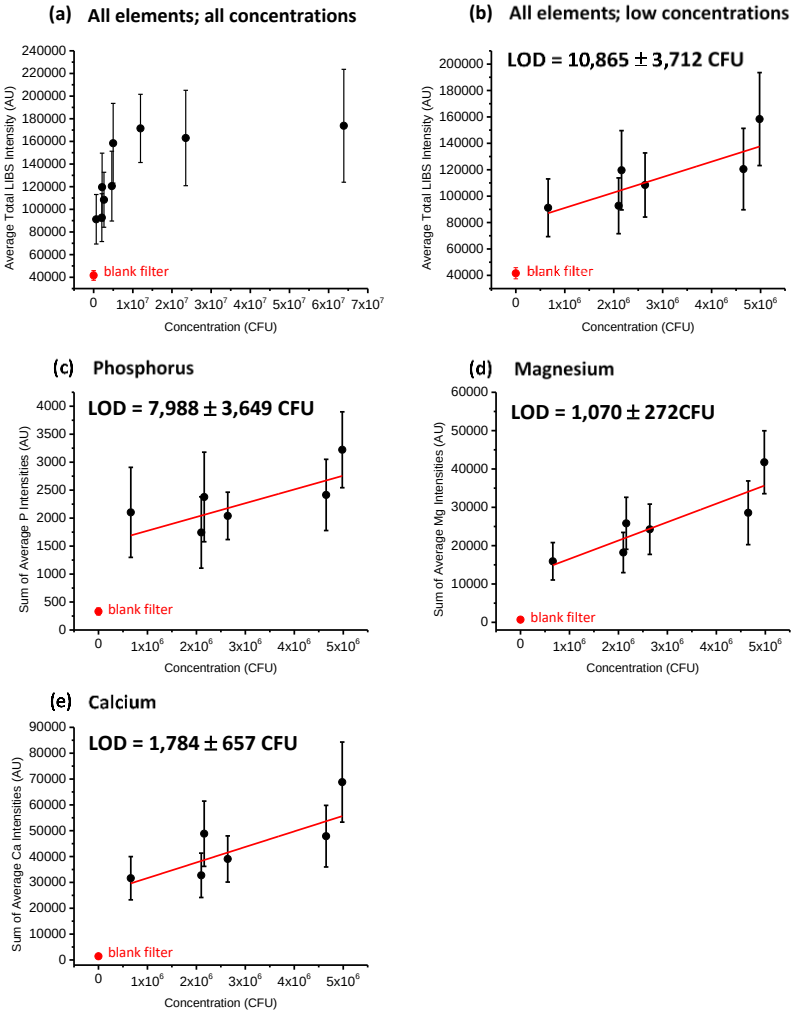
…interested graduate students always needed!
(Maybe you want to join the team)?

Email me:
rehse@uwindsor.ca

Thank you!

Using filters, a better way: the centrifuge insert

Remember?



A calibration curve constructed from forty spectra obtained from each of the nine different concentrations returned a LIBS bacterial limit of detection of 10,865 ± 3,712 CFU per laser ablation event for bacteria deposited on filters using the metal cone. Limits of detection 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 and 1,784 ± 657 CFU for calcium. This represents a factor of 50 reduction in the limit of detection compared to our previously reported value.

Bacterial LOD

Tungsten powder, average particle size of 12 μm, was used to simulate a contaminant. Tungsten powder was chosen for its inertness, cost and availability, safety of use, ease of preparation, biologically relevant size, and elemental purity.

Tungsten powder was added to a suspension of *E. coli*, vortexed, and ~ 0.1 mL was pipetted into the top of the insert prior to centrifugation.

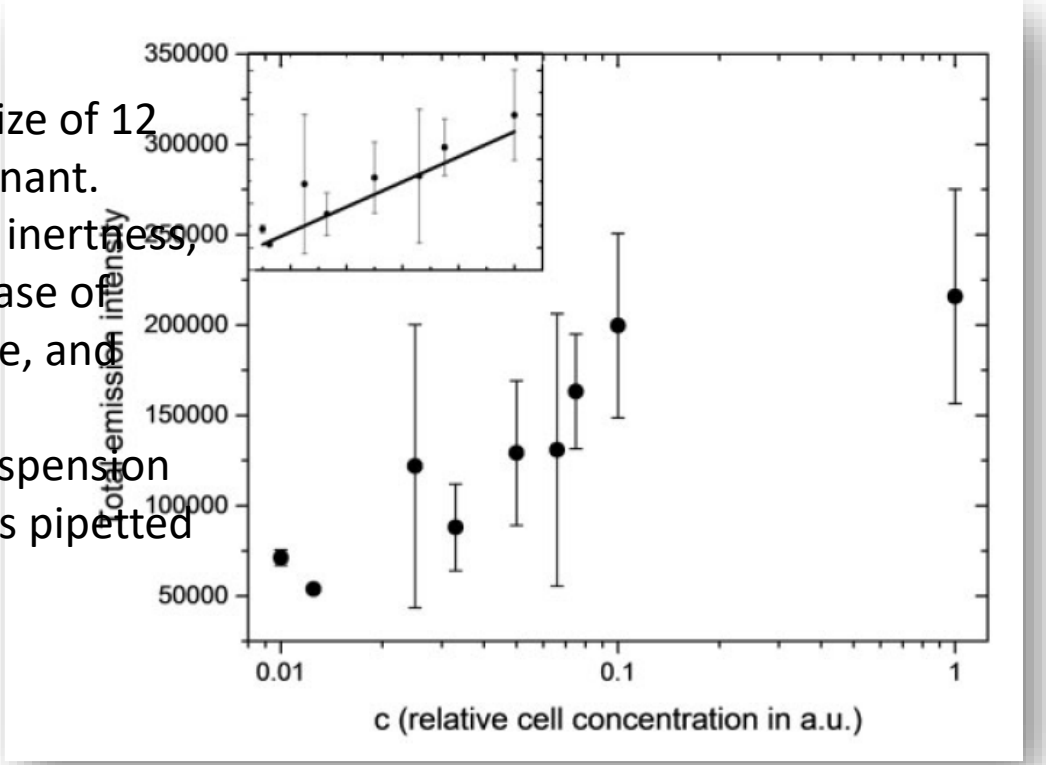
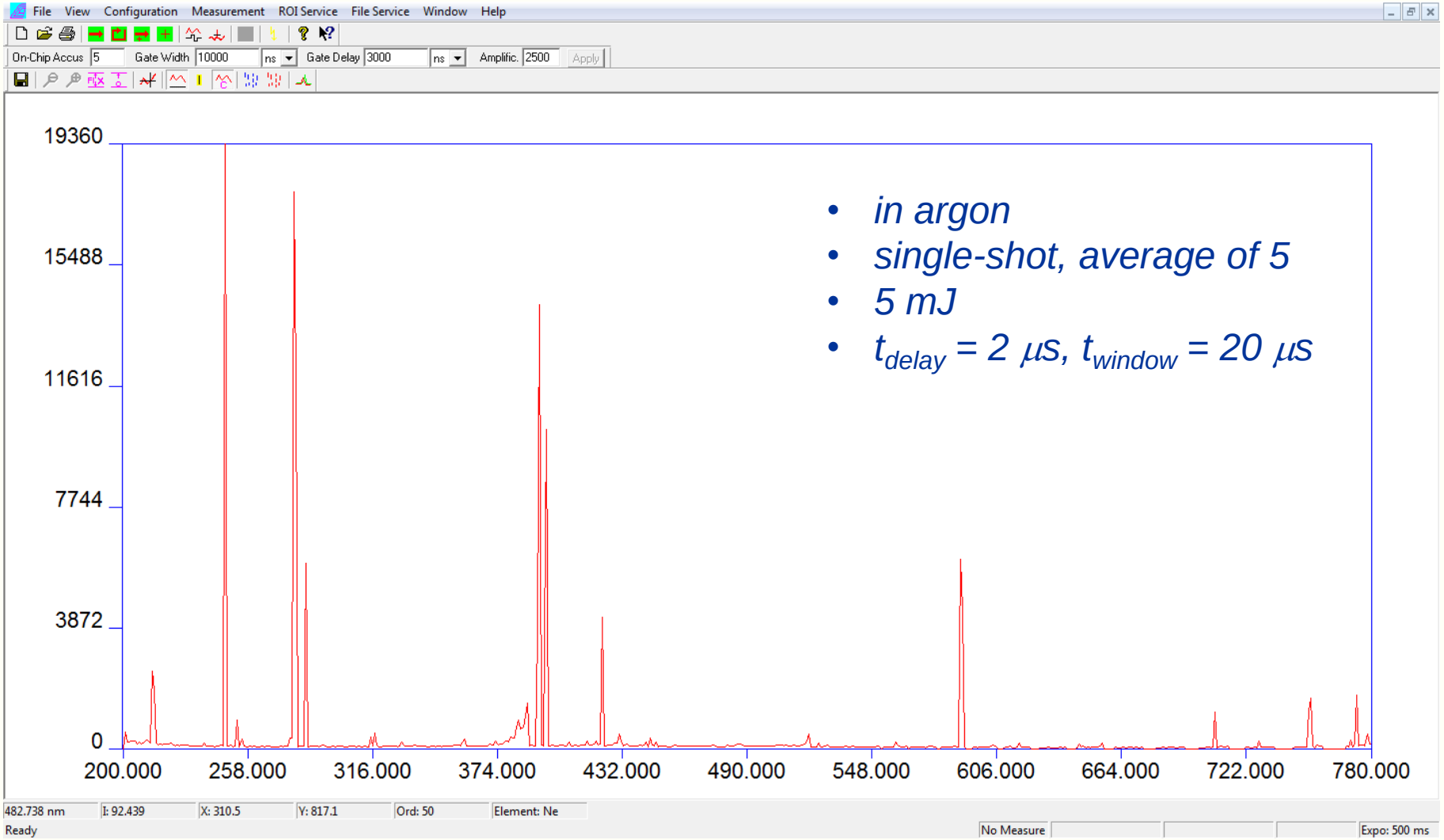


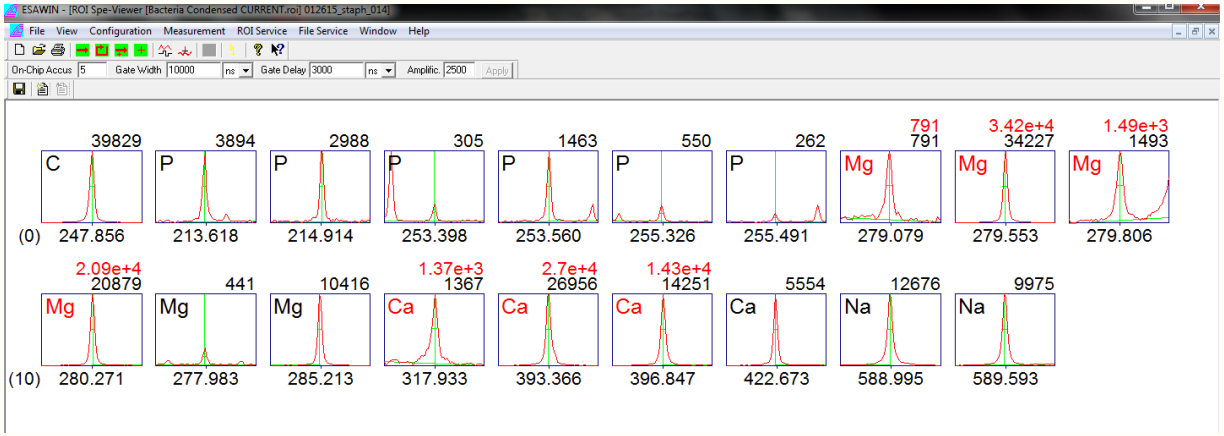
Figure 6. A log-lin calibration curve for bacterial samples. A concentration of c = 1 corresponded to 10^{11} cells/mL as determined by optical densitometry resulting in approximately 10^6 cells per ablation. This was the concentration achieved by transferring 24 h of growth for *E. coli* from a TSA plate to 1.5 mL distilled water. The inset shows a linear fit to the lowest eight concentrations on a lin-lin plot.

Derek J. Gillies, and Steven J. Rehse, Applied Spectroscopy 2016, Vol. 70(3) 485–493

Typical Bacterial Spectrum

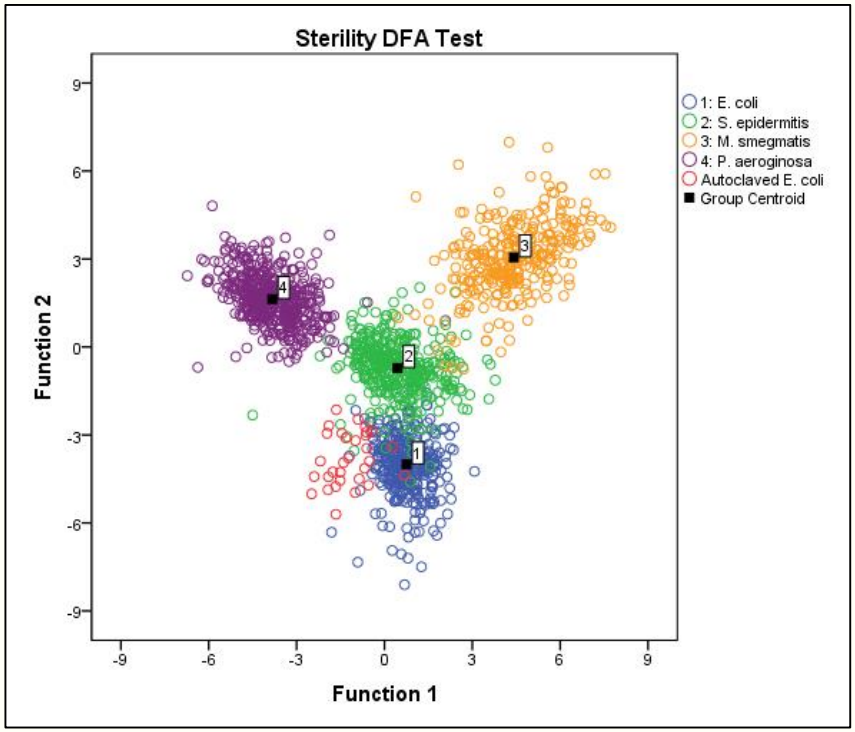


Variable Down-Selection



- New classification model
- 164 independent variable
  - 19 line intensities (all divided by sum)
  - 145 ratios of intensities
- No other metals. Beware?
  - Farooq (2014) sees S, Cl, Mn, Fe, Al, Cu, etc.
  - Sivakumar (2015) only sees Ca, Na, Mg, K, O, H, C, P
  - We see Ni, Fe, Ti only when contaminated!

Performance With New Library



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Escherichia	TRUE	FALSE	*Staphylococcus*	TRUE	FALSE
Positive	98.28%	0.77%	Positive	97.75%	1.44%
Negative	99.23%	1.72%	Negative	98.56%	2.25%
Mycobacterium	**TRUE**	**FALSE**	***Pseudomonas***	**TRUE**	**FALSE**
Positive	95.36%	0.33%	Positive	99.57%	0.22%
Negative	99.67%	4.64%	Negative	99.78%	0.43%

Sensitivity: 98 ± 2% Specificity: 99 ± 1%

PLS-DA Classification Grouped by Species

Escherichia	TRUE	FALSE	*Staphylococcus*	TRUE	FALSE
Positive	96.55%	1.12%	Positive	96.75%	1.53%
Negative	98.88%	3.45%	Negative	98.47%	3.25%
Mycobacterium	**TRUE**	**FALSE**	***Pseudomonas***	**TRUE**	**FALSE**
Positive	97.02%	0.41%	Positive	98.92%	0.33%
Negative	99.59%	2.98%	Negative	99.67%	1.08%

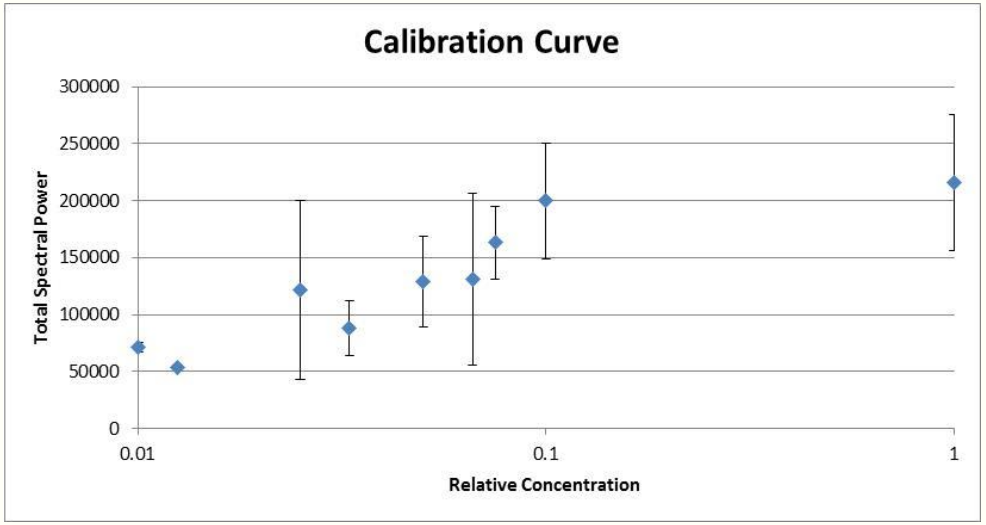
Sensitivity: 97 ± 3% Specificity: 99 ± 2%

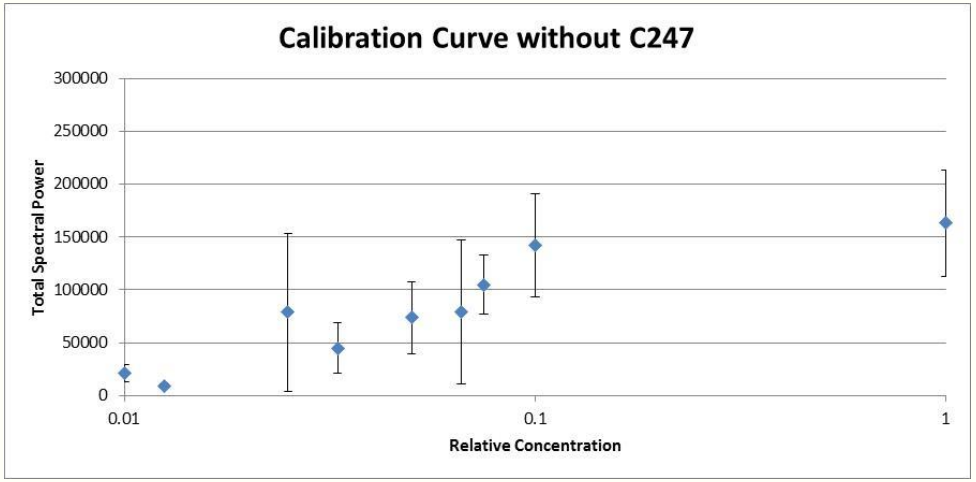
All external validation results

	DFA (by filter)	DFA (by species) above	PLSDA (by species) above
Sensitivity	0.93±0.07	0.98±0.02	0.97±0.03
Specificity	0.98±0.03	0.99±0.01	0.99±0.02

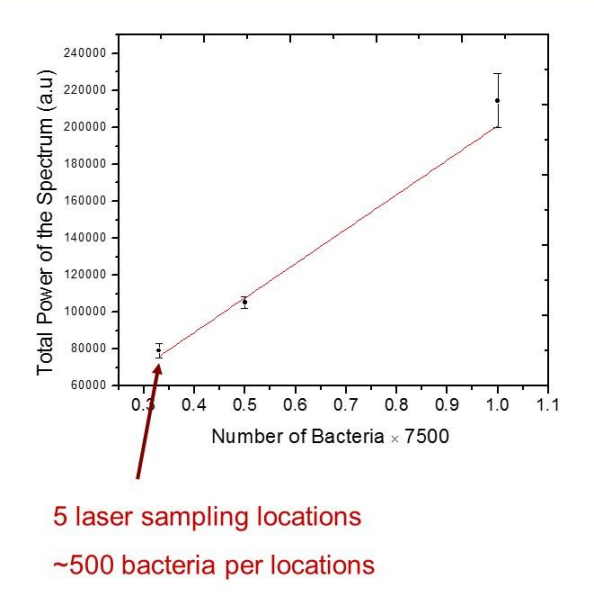
"by filter" means approximately 30 groups in DFA, no relationships between groups assumed

New Concentration Study

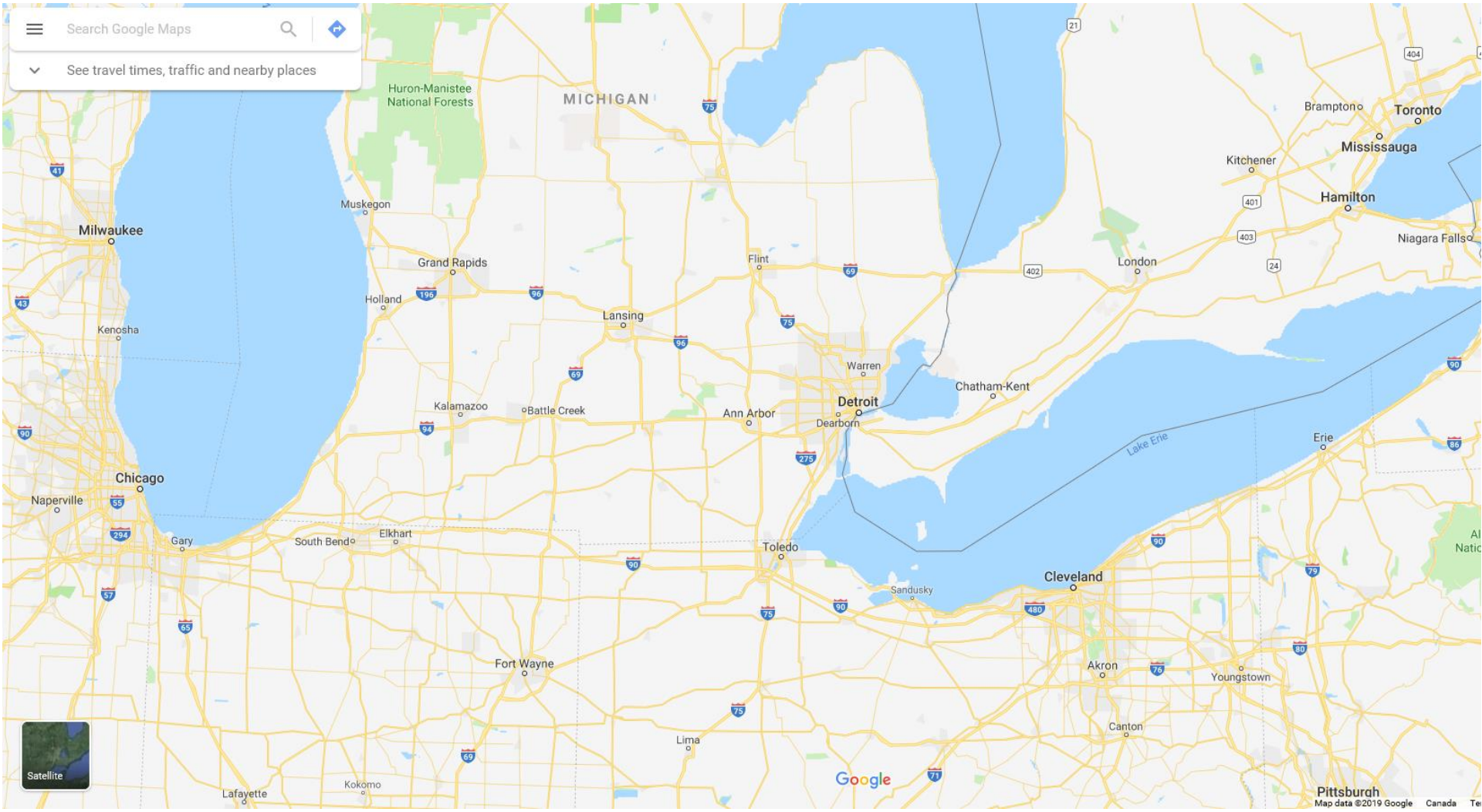




Previous result



- Performed with serial dilutions.
- "Concentration 1" → harvest entire plate of colonies off TSA, suspend in 1.5 mL distilled H_20
- Measure with optical densitometry
- OD=0.1 measured for C=0.001 (from literature OD 0.1=10^8 cells/mL).
- C=1 → 10^{11} cells/mL
- Implies for C=1, 10^6/shot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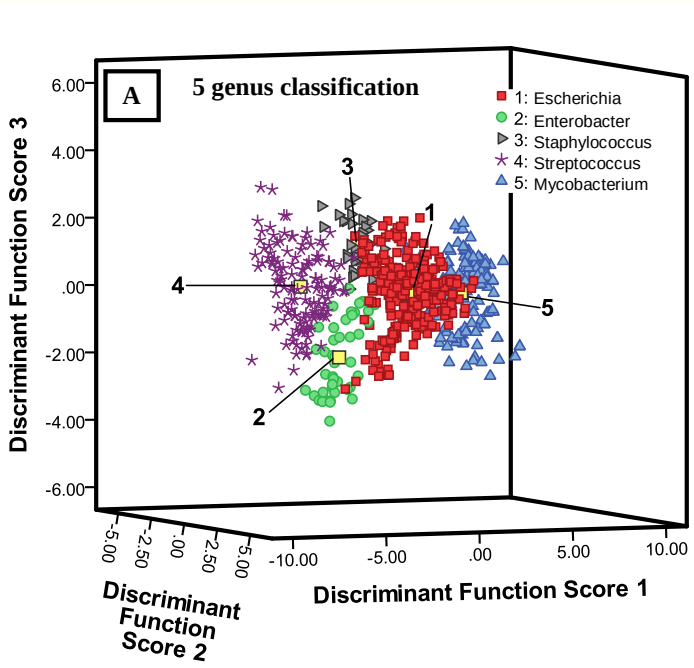
How unique is “unique”?

- ✓ 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

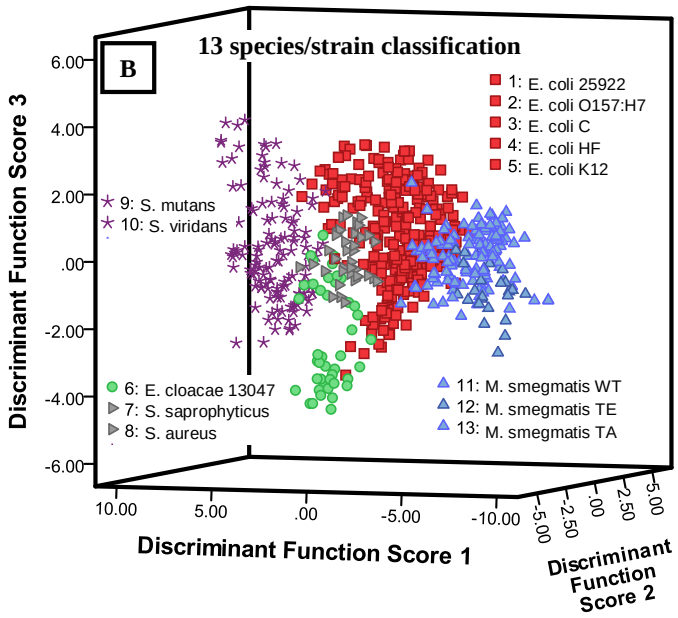
E. COLI	True	False
Positive	95.65%	9.17%
Negative	90.83%	4.35%
STAPHYLOCOCCUS	**True**	**False**
Positive	54.05%	0.51%
Negative	99.49%	45.95%
STREPTOCOCCUS	**True**	**False**
Positive	95.59%	1.02%
Negative	98.98%	4.41%
MYCOBACTERIUM	**True**	**False**
Positive	88.31%	1.06%
Negative	98.94%	11.69%

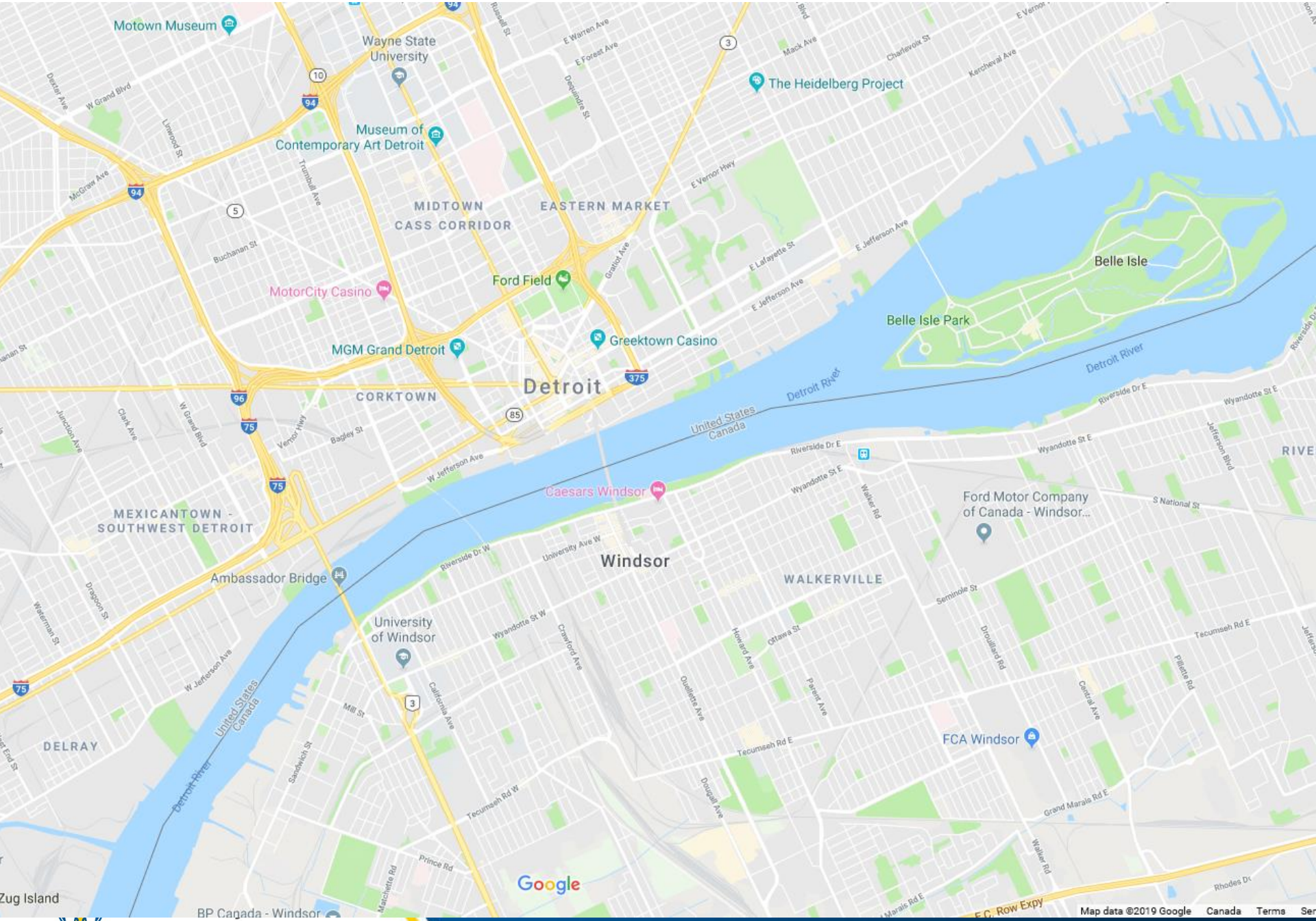
DFA

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 %

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