Biomedical and Biological Applications of Laser-Induced Breakdown Spectroscopy in Clinically Relevant Systems

FACSS PRESENTS

SCIX 2018

18LIBS03: Biomedical and Pharmaceutical Applications

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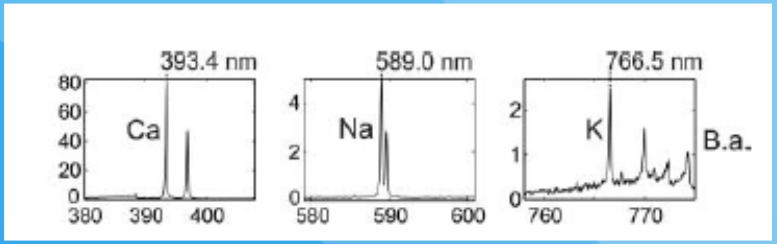
Staph. epidermidis

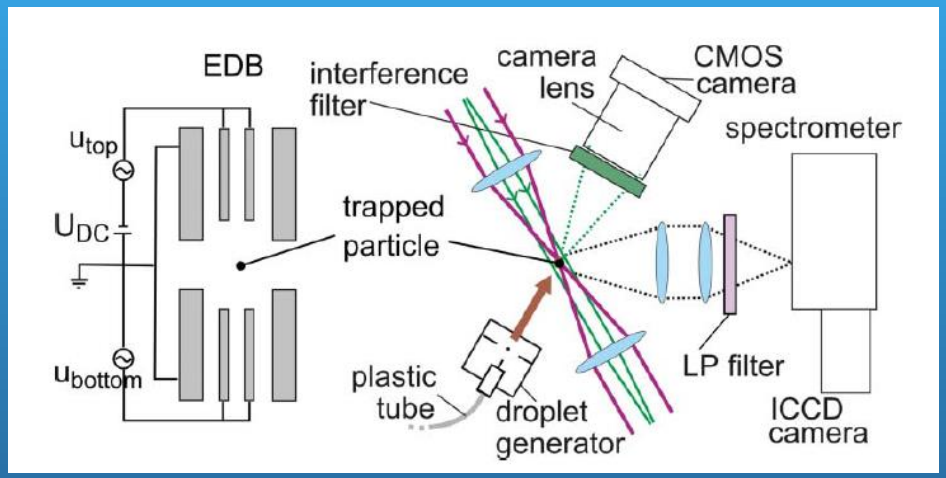
What is New Bacteriological Identification?

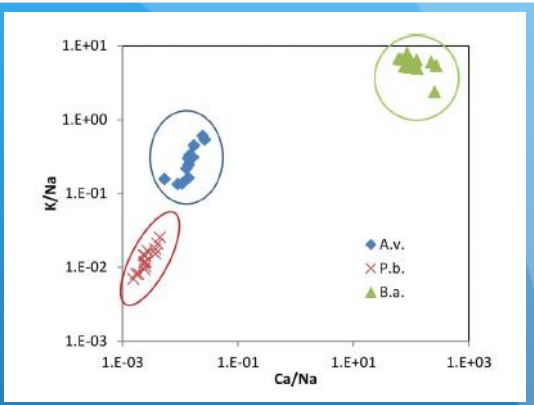
- Three of the most recent papers in the field have been investigating hyphenated techniques

Good SNR from a single spore
(*shown by Dixon and Hahn in 2005*.)

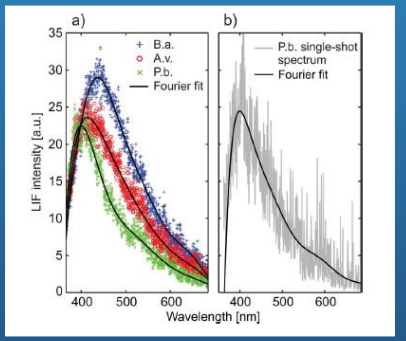


355 nm LIBS with 9 mJ



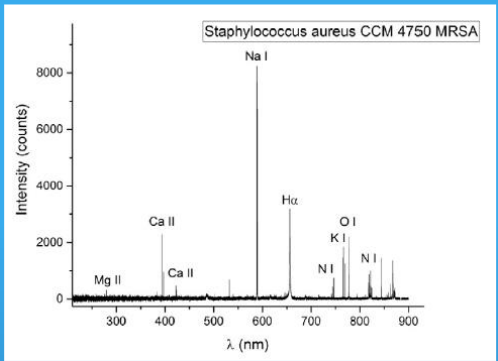


LIF distinguishes bioaerosols from other aerosols



Identification of single microbial particles using electro-dynamic balance assisted laser-induced breakdown and fluorescence spectroscopy

S. Saari, S. Jarvinen, T. Reponen, J. Mensah-Attipoe, P. Pasanen, J. Toivonen, and J. Keskinen
Department of Physics, Tampere University of Technology, Tampere, Finland;
Department of Environmental Science, University of Eastern Finland, Kuopio, Finland;
Department of Environmental Health, University of Cincinnati, Cincinnati, Ohio, USA
Aerosol Science and Technology, 50:2, 126-132, 2016.



532 nm LIBS with 50 mJ

Fig. 1. *Staphylococcus aureus* CCM 4223 (S aur) - after measurement.

PCA and self-organizing maps on merged data

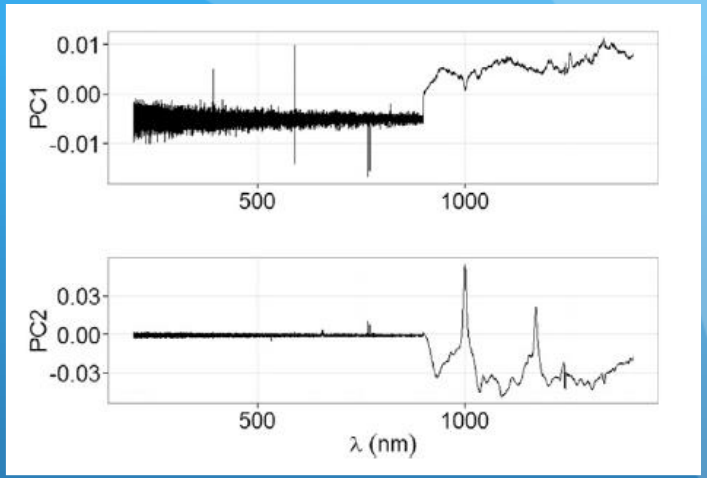


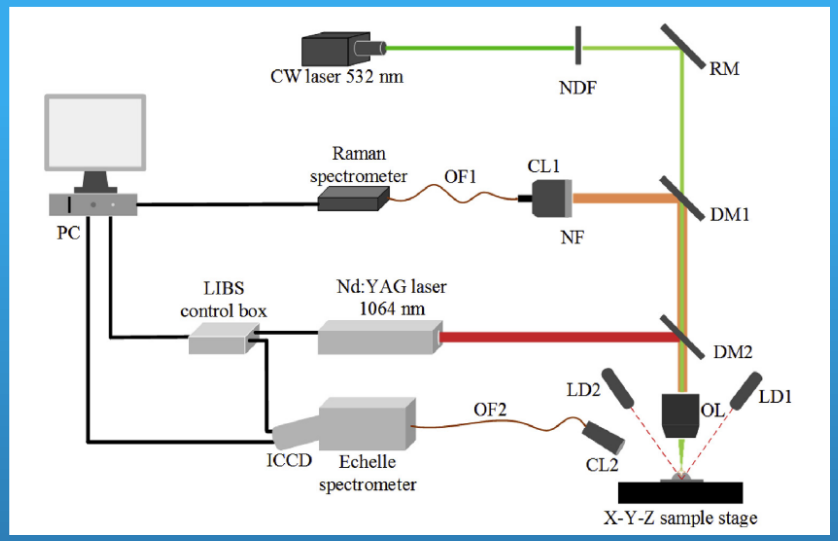
Table 2
Classification success rate for each sample and each dataset respectively.

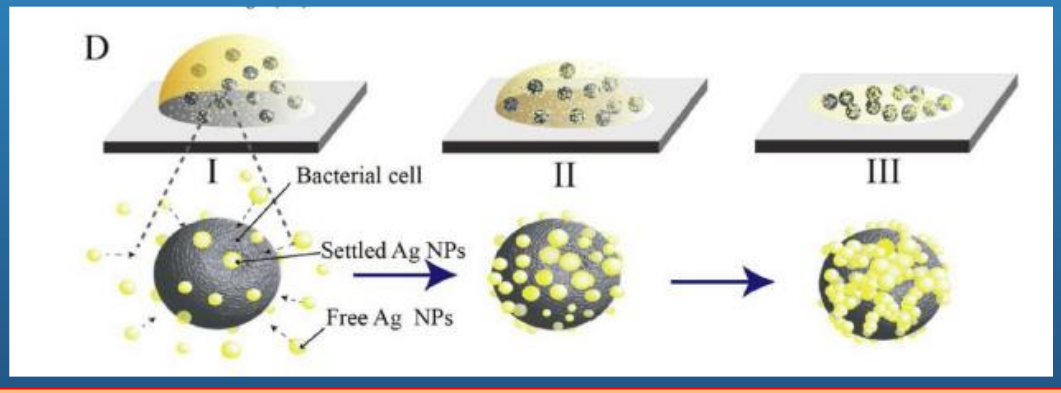
Bacteria strain/classification success	LIBS	Raman	Merged data
Staphylococcus pseudointermedius (S pse)	70%	50%	100%
Staphylococcus aureus CCM 4750 - methicillin resistant (MRSA)	45%	75%	100%
Staphylococcus aureus CCM 3953 - methicillin sensitive (MSSA)	75%	100%	100%
Escherichia coli CCM 3954 (E coli)	100%	100%	100%
Staphylococcus sciuri (S sci)	100%	100%	100%
Staphylococcus aureus CCM 4223 (S aur)	100%	100%	100%

Combination of laser-induced breakdown spectroscopy and Raman spectroscopy for multivariate classification of bacteria

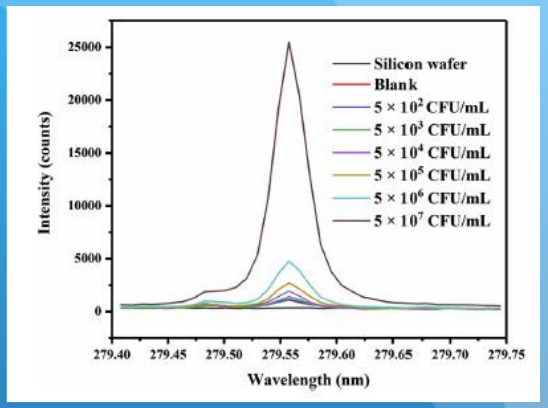
D. Prochazka, M. Mazura, O. Samek, K. Rebrošová, P. Pořízka, J. Klus, P. Prochazková, J. Novotný, K. Novotný, J. Kaiser
Central European Institute of Technology, Brno University of Technology, Purkyňova 123, CZ 61200, Brno, Czech Republic
Spectrochimica Acta Part B 139 (2018) 6–12

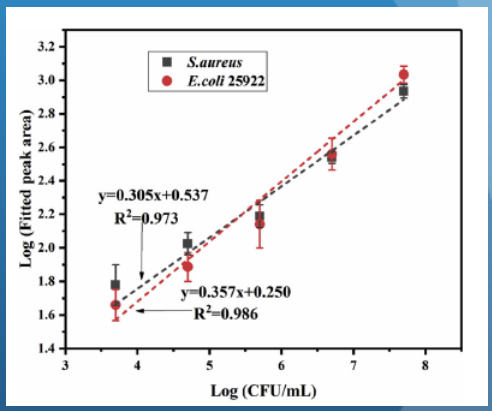
1064nm LIBS with 30 mJ





LIBS not used for identification, but for quantification
(*shown by Rehse et al. in 2010.*)

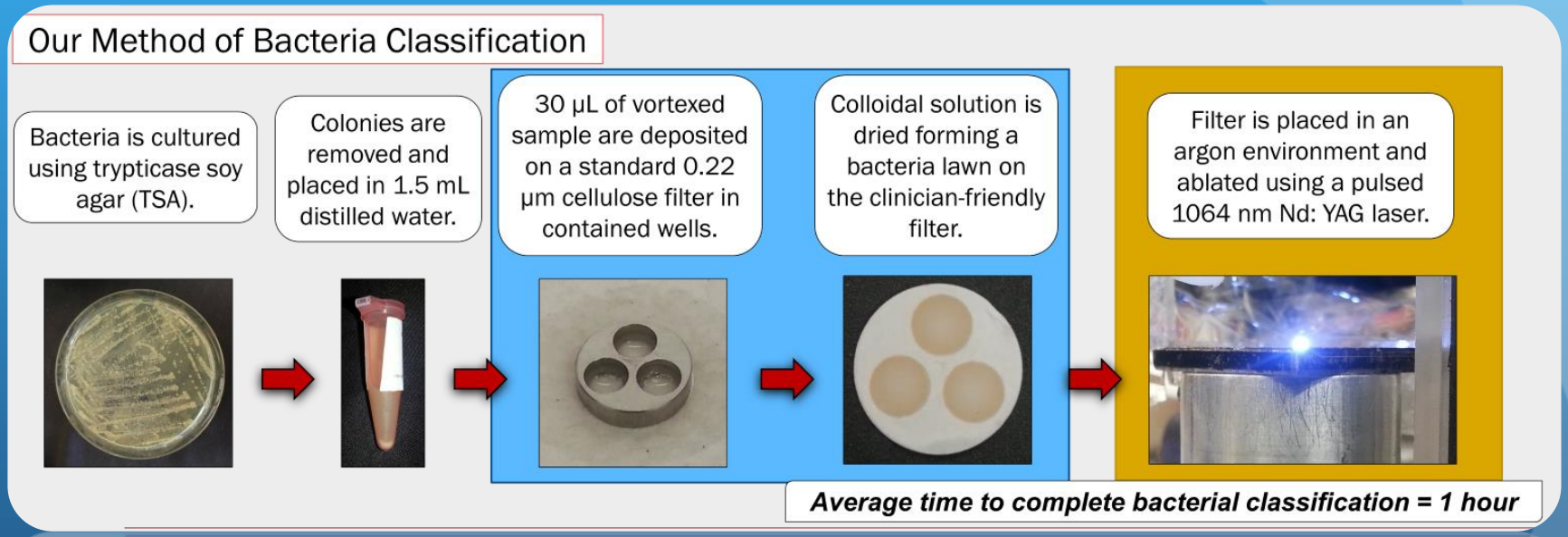




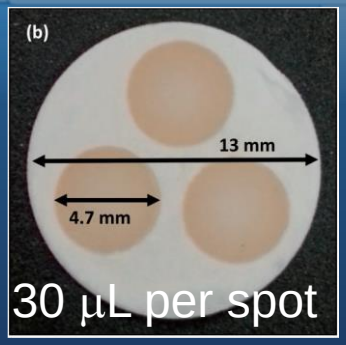
A novel strategy for rapid detection of bacteria in water by the combination of three-dimensional surface-enhanced Raman scattering (3D SERS) and laser induced breakdown spectroscopy (LIBS)

W. Liao, Q. Lin, S. Xie, Y. He, Y. Tian, Y. Duan
School of Chemical Engineering, Sichuan University, Chengdu, 610065, PR China
Analytica Chimica Acta, Available online 26 June 2018

Conversely…we've been trying to make the preparation faster/easier

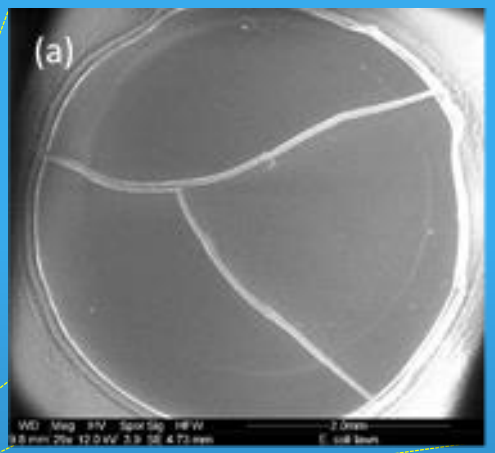


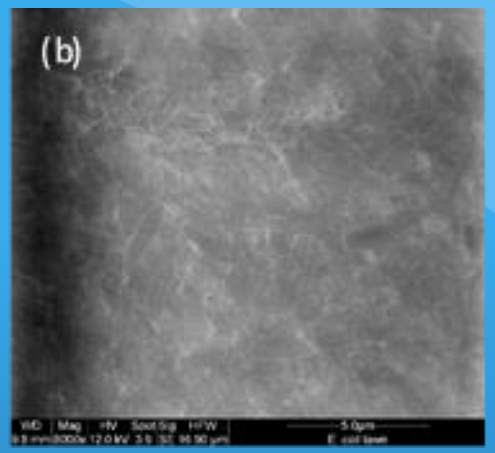
>10^9 cfu/ml

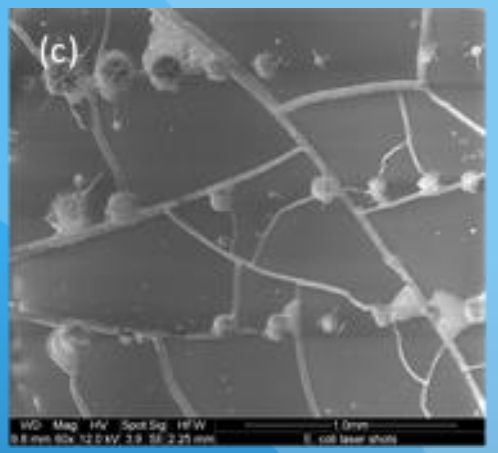


This is a LOT of bacteria!

2014-2016

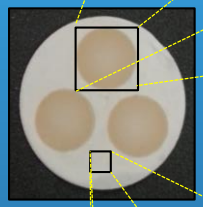


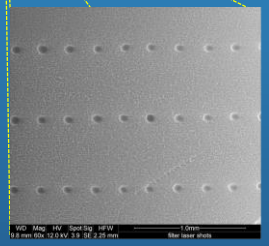


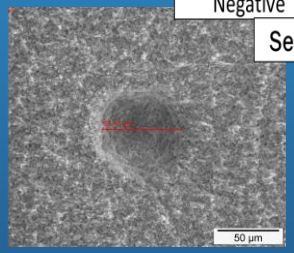


(a) DFA

(b) PLS-DA







Escherichia	TRUE	FALSE
Positive	98.28%	0.77%
Negative	99.23%	1.72%

Staphylococcus	TRUE	FALSE
Positive	97.75%	1.44%
Negative	98.56%	2.25%

Mycobacterium	TRUE	FALSE
Positive	95.36%	0.33%
Negative	99.67%	4.64%

Pseudomonas	TRUE	FALSE
Positive	99.57%	0.22%
Negative	99.78%	0.43%

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

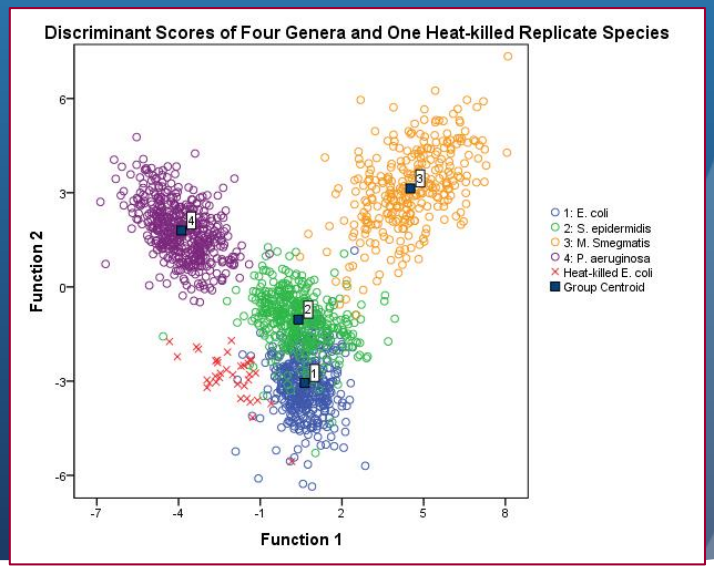
Escherichia	TRUE	FALSE
Positive	96.55%	1.12%
Negative	98.88%	3.45%

Staphylococcus	TRUE	FALSE
Positive	96.75%	1.53%
Negative	98.47%	3.25%

Mycobacterium	TRUE	FALSE
Positive	97.02%	0.41%
Negative	99.59%	2.98%

Pseudomonas	TRUE	FALSE
Positive	98.92%	0.33%
Negative	99.67%	1.08%

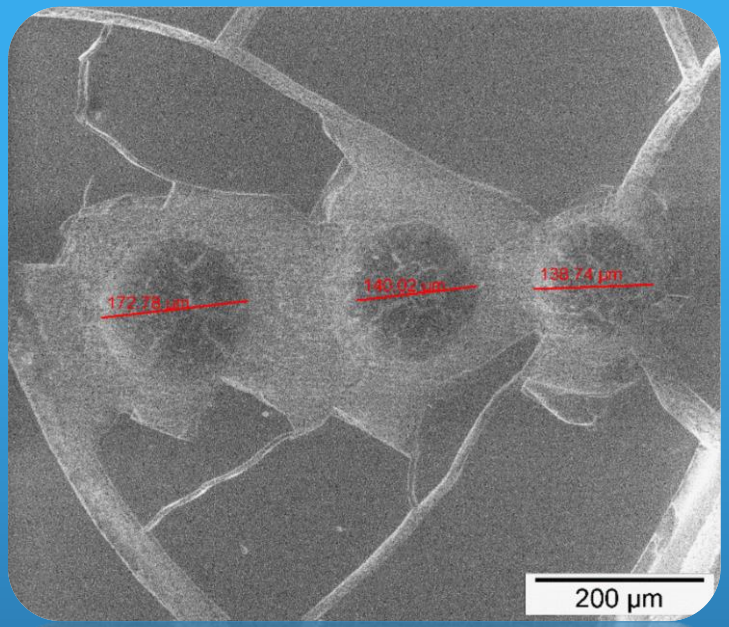
Sensitivity: 97 ± 3% Specificity: 99 ± 2%



Highly efficient discrimination still possible on nitrocellulose medium

DFA and PLS-DA perform similarly

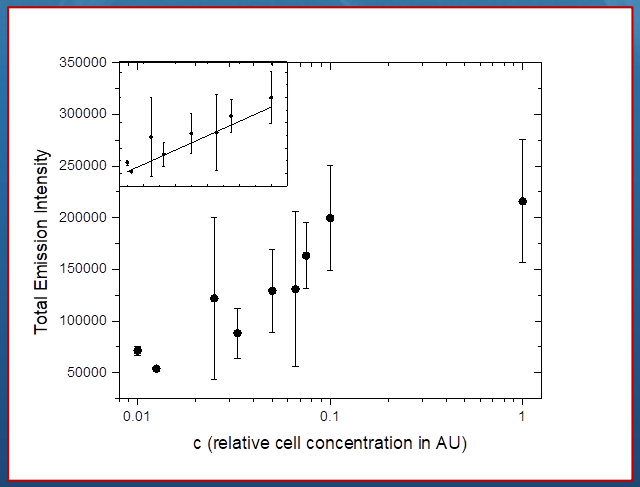
2014-2016

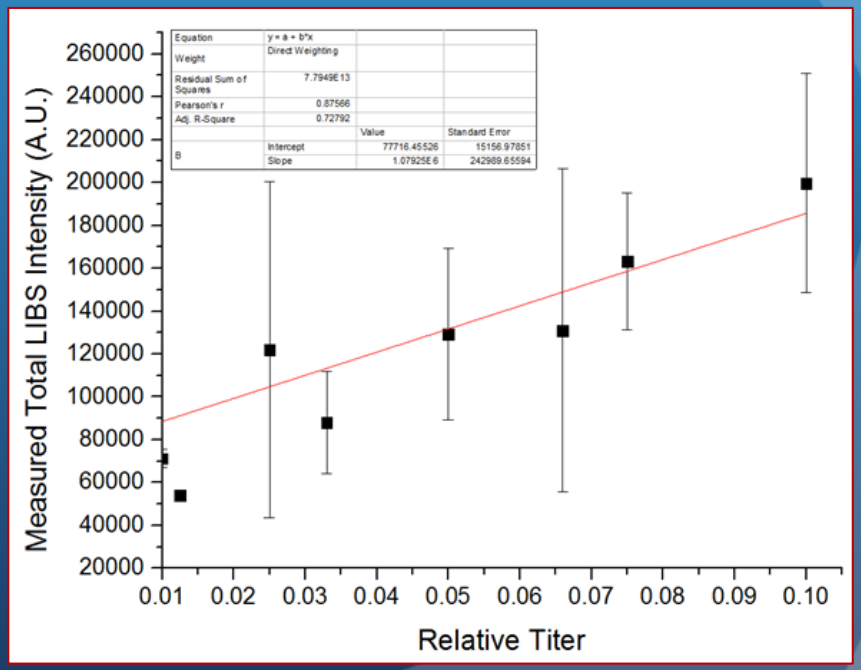


- ✓ Know ablation area
- ✓ Know bacterial titer (from absorption optical densitometry)
- ✓ Know bacterial deposition area

- ➢ **Known # cells per ablation spot**

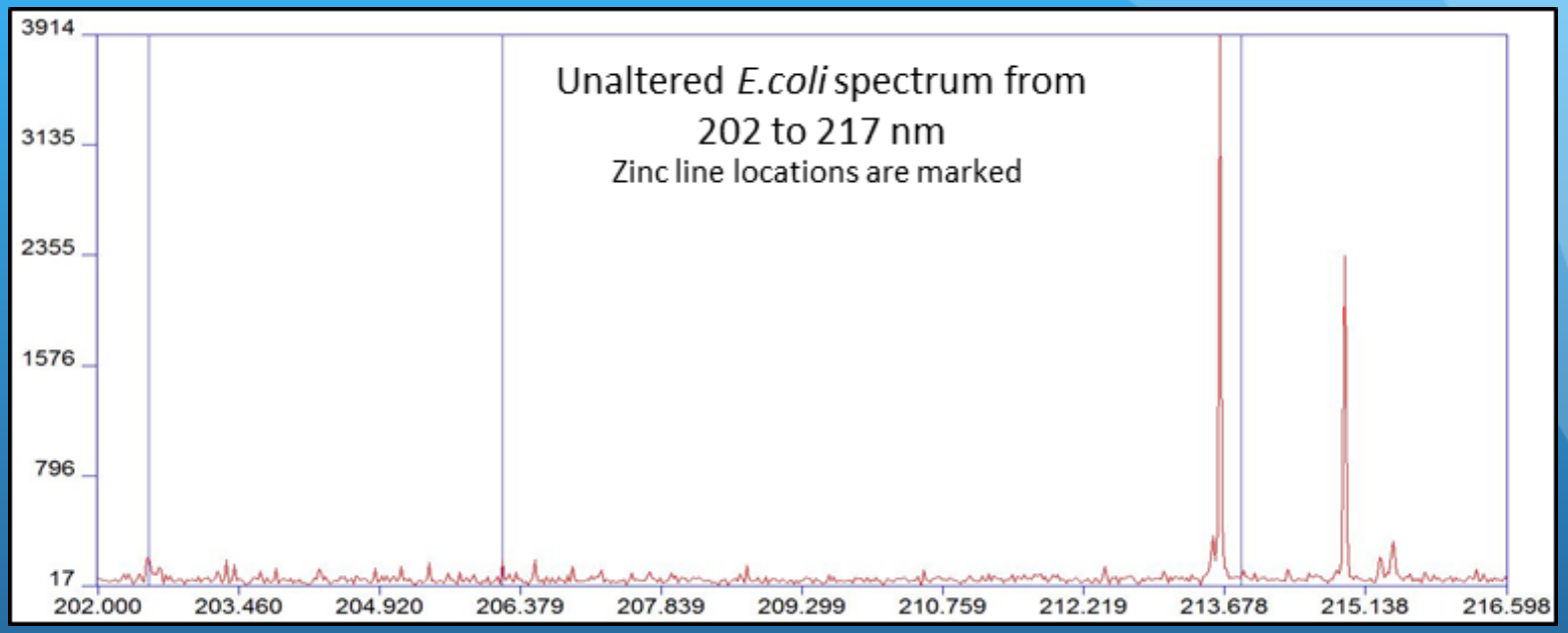
limit of detection of 48000±12000 CFU per ablation event





2014-2016

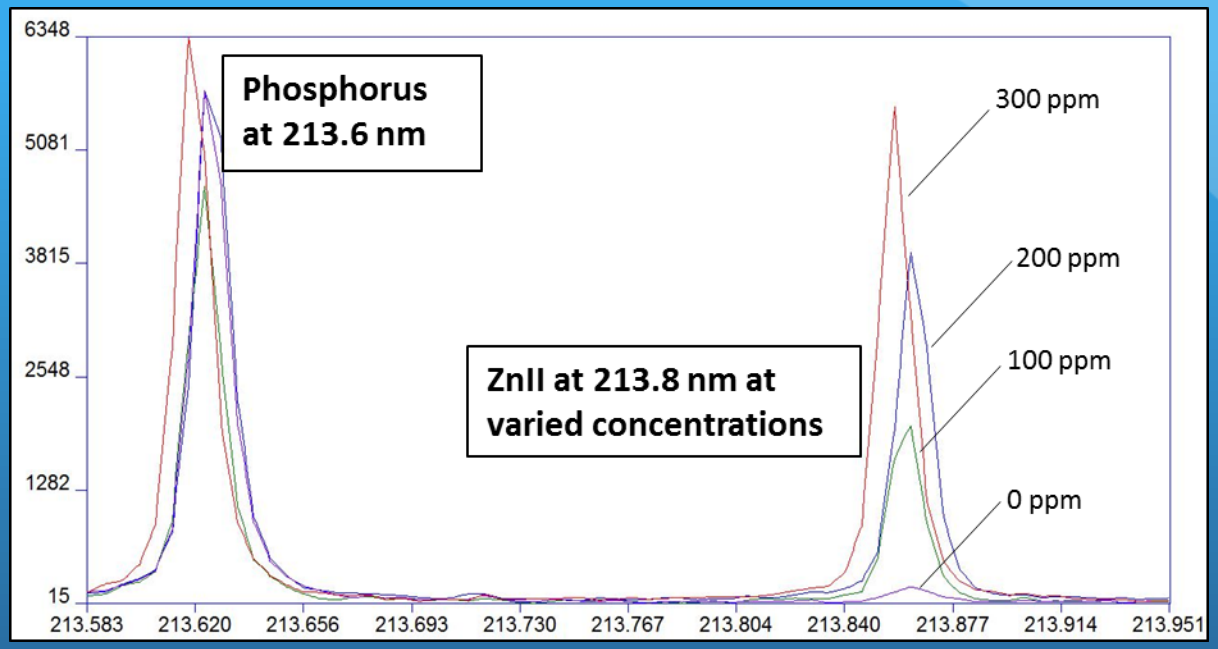
Altering Cell Metal Content: Zinc



Zinc lines are not distinguishable from noise at normal growth conditions using our testing protocol.

2016

Altering Cell Metal Content: Zinc



When zinc is added to the *E. coli* growth medium (TSA medium plates), cellular zinc is observed

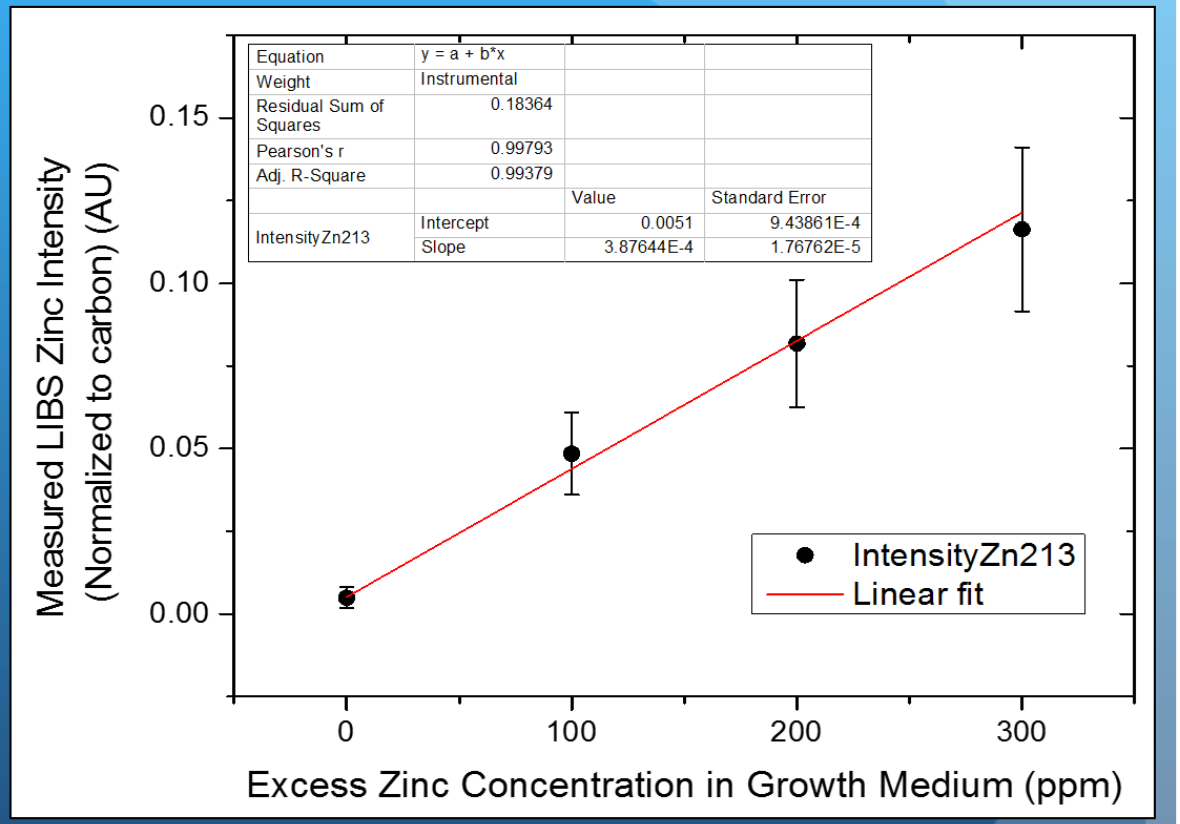
2016

Altering Cell Metal Content: Zinc

A linear fit of zinc line intensity to the excess zinc concentration gives an adjusted r^2 of 0.994.

The limit of detection (LOD) as calculated from this fit is 11 ppm.

The maximum concentration allowable for drinking water is 5 ppm.



Environmental Application

Since bacterial species take their nutrients from their environment, bacteria have been used as an indicator of environmental health, with trace metals in the cells being indicative of contamination of a water supply.

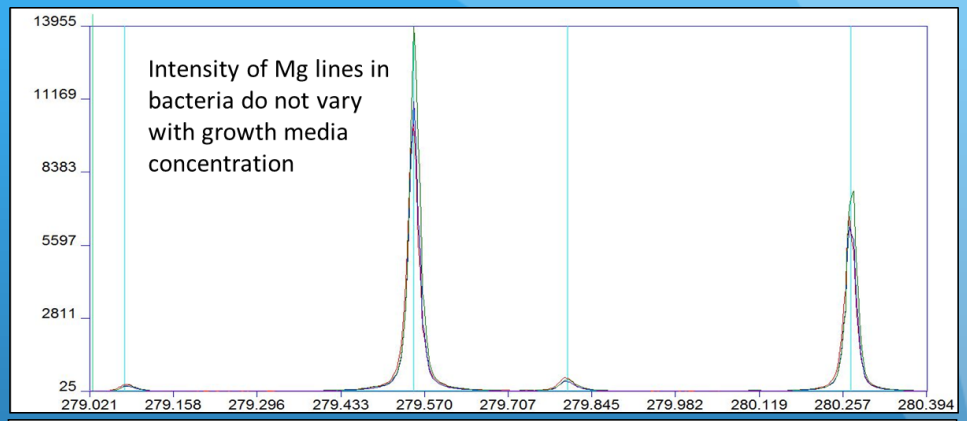
2016

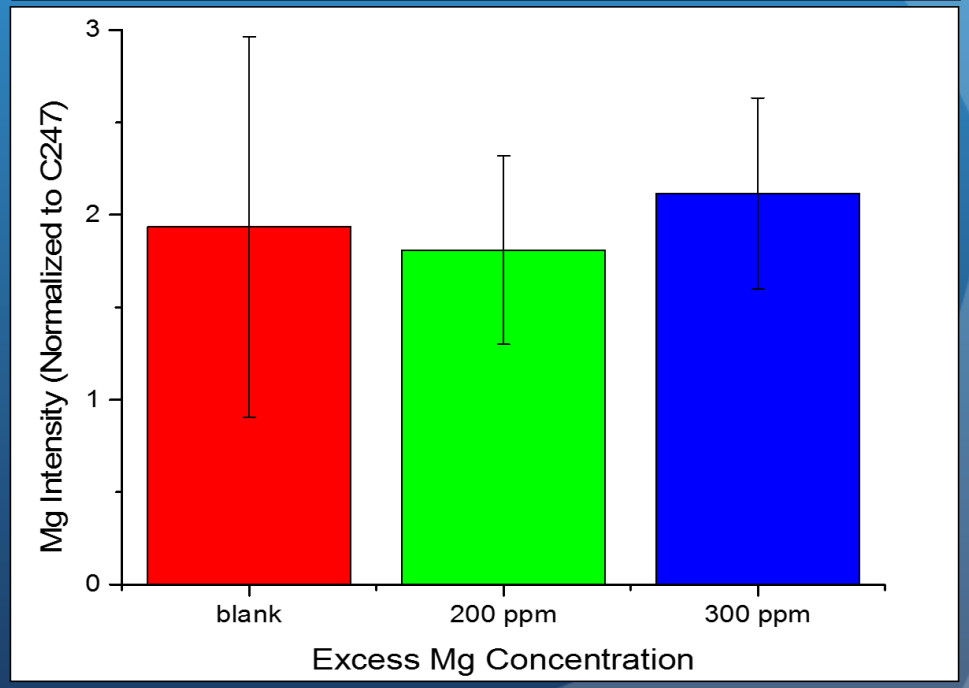
Altering Cell Metal Content: Magnesium

As excess Mg was added to the growth medium, the intensity of the Mg emission lines was largely unchanged.

The deviation in intensity reduced as the surplus increased.

A sample was prepared wherein Mg was precipitated out of the agar solution using HCl prior to autoclaving. This plate provided no bacterial growth.





2016

New Mounting Procedure: Concentration by Centrifugation

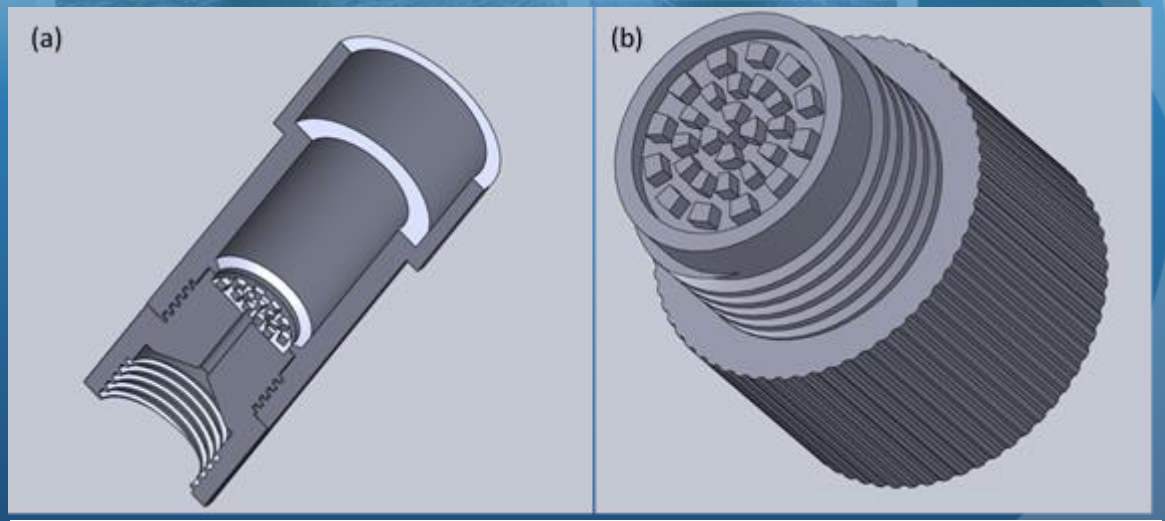
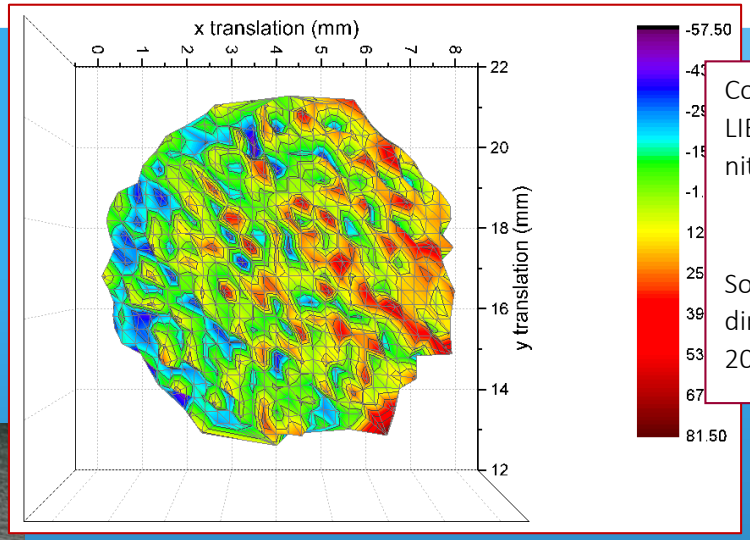


Figure 6.1: (a) Full centrifuge insert design in cross section. Filter paper is placed on the male end (b) of the device, and a seal is produced by the pressure generated by the threads. Pedestals under the filter paper prevent it from resting directly on a flat surface, allowing water to freely pass through the filter

Filter catches 90% - 95% of bacteria after 3 min of centrifugation (with some possible dependence on titer)

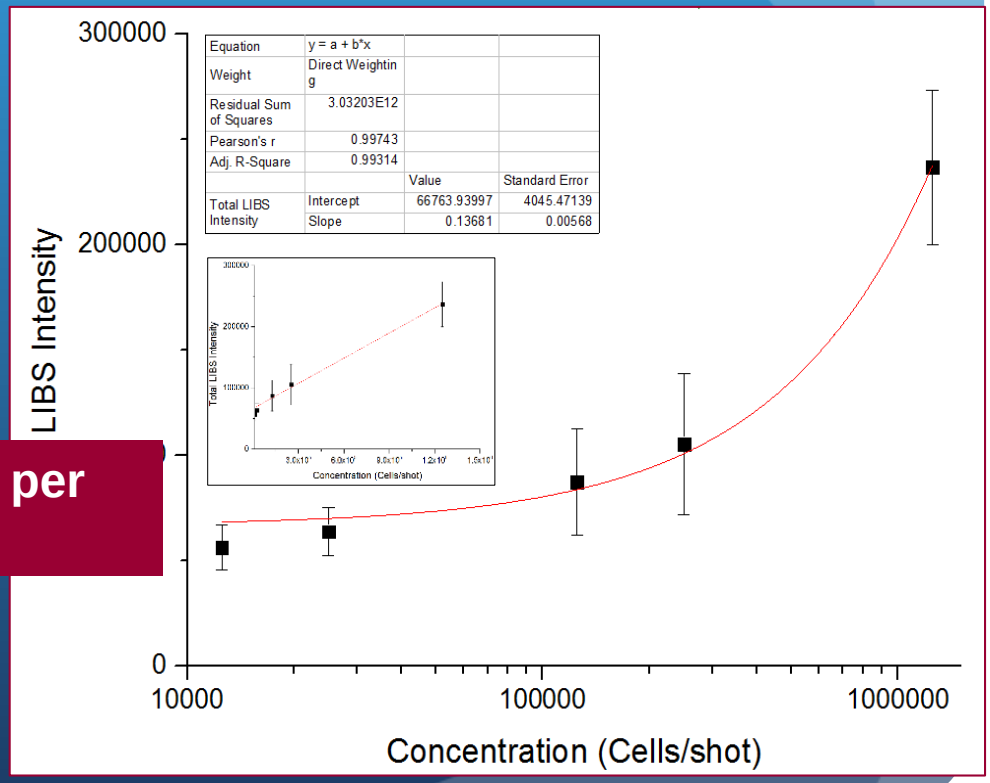
2016-2018



Colour map indicating percent difference of the total measured LIBS intensity from the average as a function of position on a nitrocellulose filter.

Some increase is observed with motion in the positive x-direction, but this increase spans from approximately -20 to 20% difference from the mean

Calibration curve for data acquired using specimens prepared with the centrifuge insert. The plot is displayed on a log-lin scale. The inset plot shows the same data on a lin-lin scale

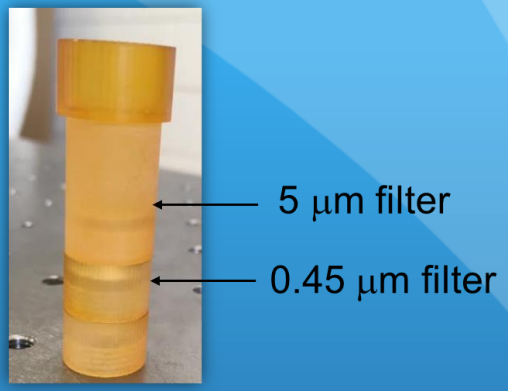


limit of detection of 60000±5000 CFU per ablation event

2016

New Mounting Procedure: Isolation by Dual-Stage Filtration

Dual stage centrifugation insert prototype



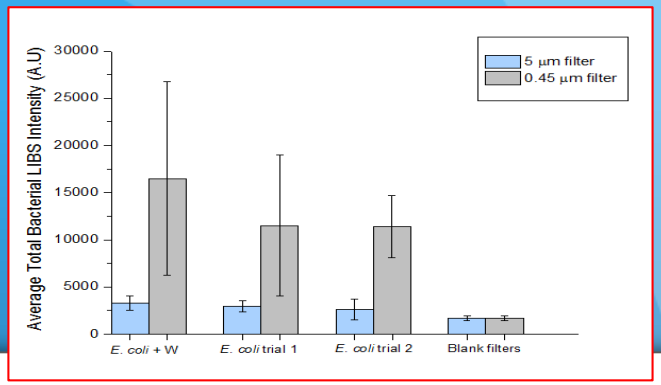
An *E. coli* suspension with tungsten powder (12 μm APS) as the contaminant was deposited in the insert with the 5 μm filter paper on top and the 0.45 μm filter paper below it.

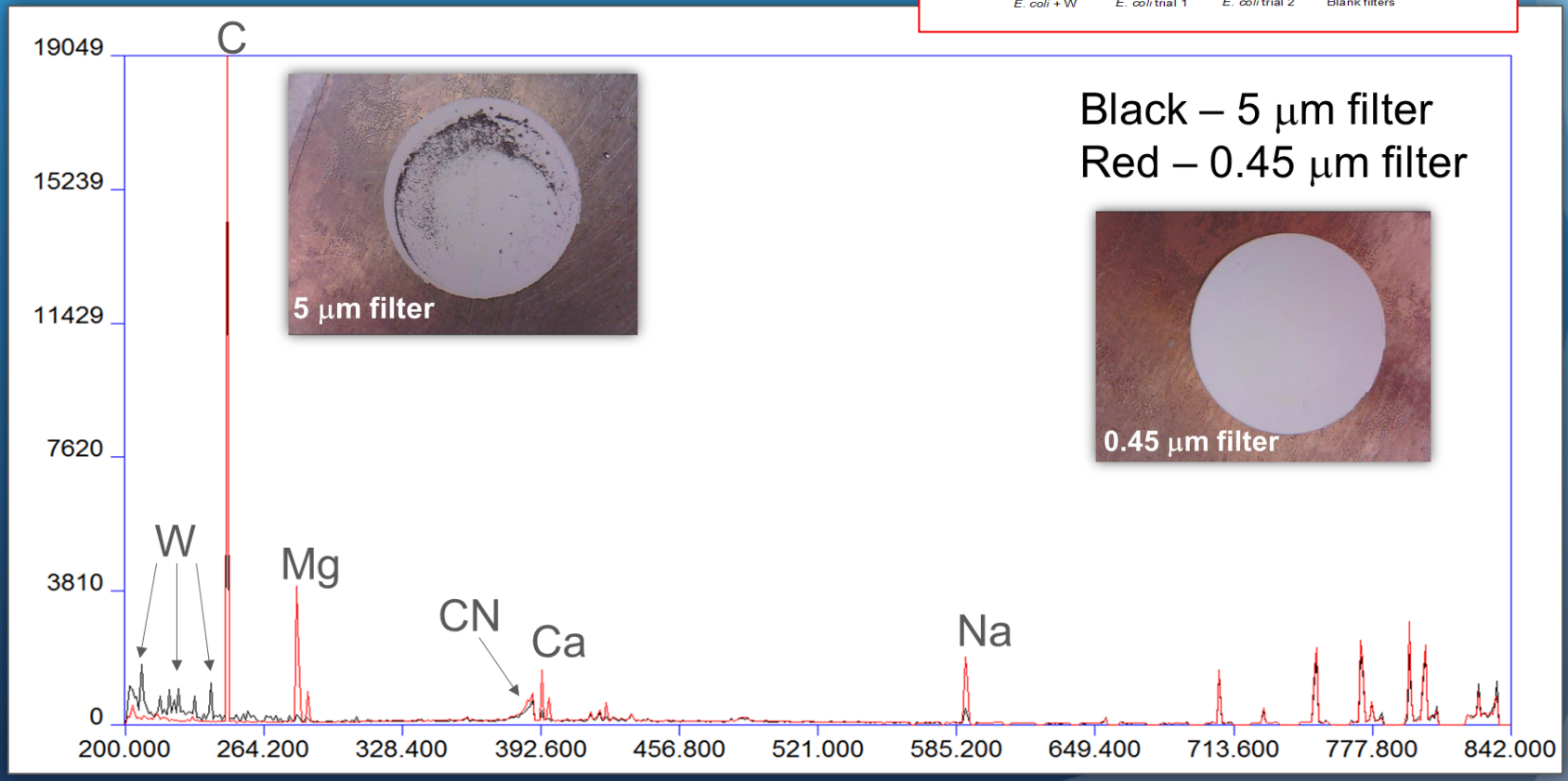
The tungsten powder was caught by the 5 μm filter while 90% of the bacteria passed through it and settled onto the 0.45 μm filter.

2017

New Mounting Procedure: Isolation by Dual-Stage Filtration

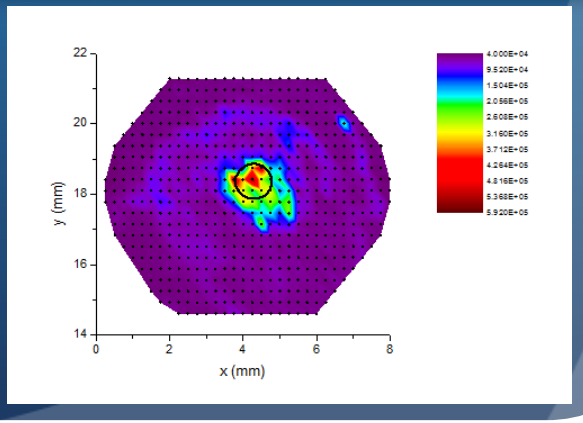
90% of the bacteria passed through 5 μm and settled onto the 0.45 μm filter.





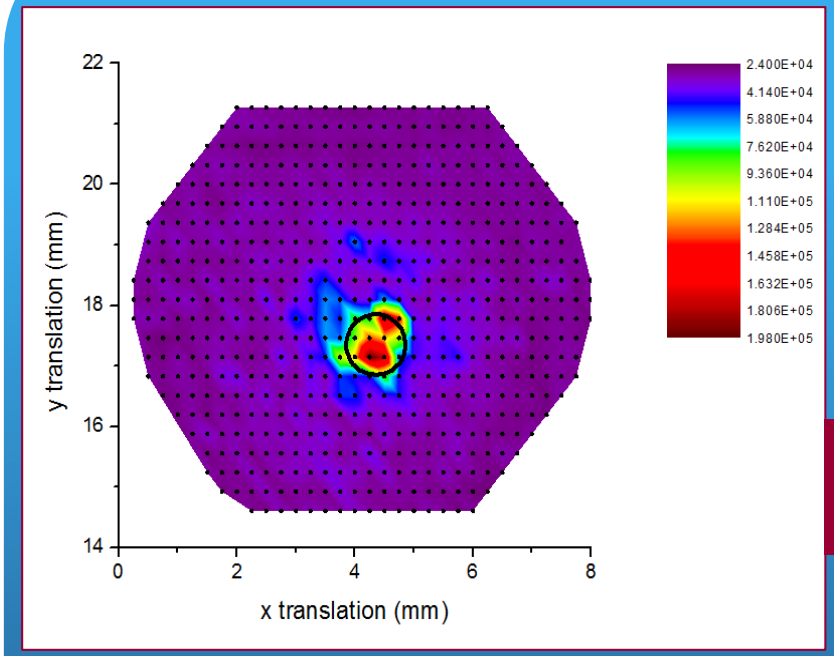
New Mounting Procedure: Concentration by Cone

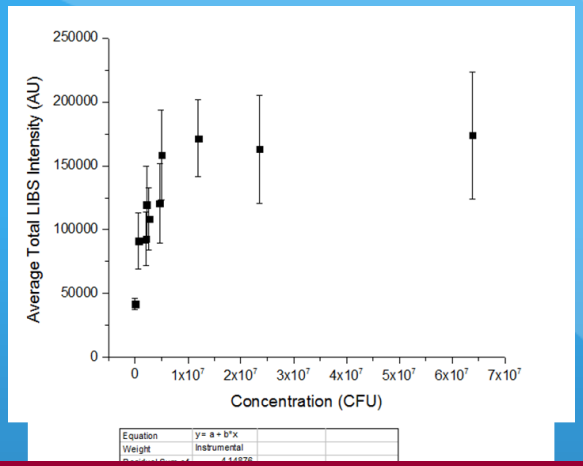
To concentrate all the bacteria into one spot (one laser shot) a custom funnel was constructed for our centrifuge insert



Each point on the map corresponds to a single laser shot, and the color indicates the LIBS bacterial intensity, with purple indicating no LIBS bacterial signal, and red indicating the region with the strongest LIBS bacterial signal.

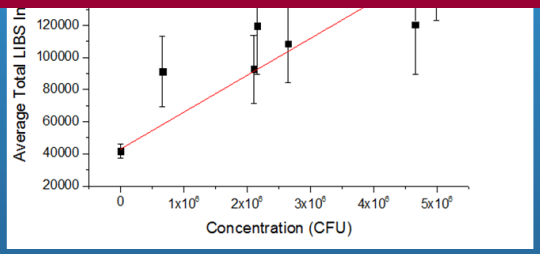
2017-2018



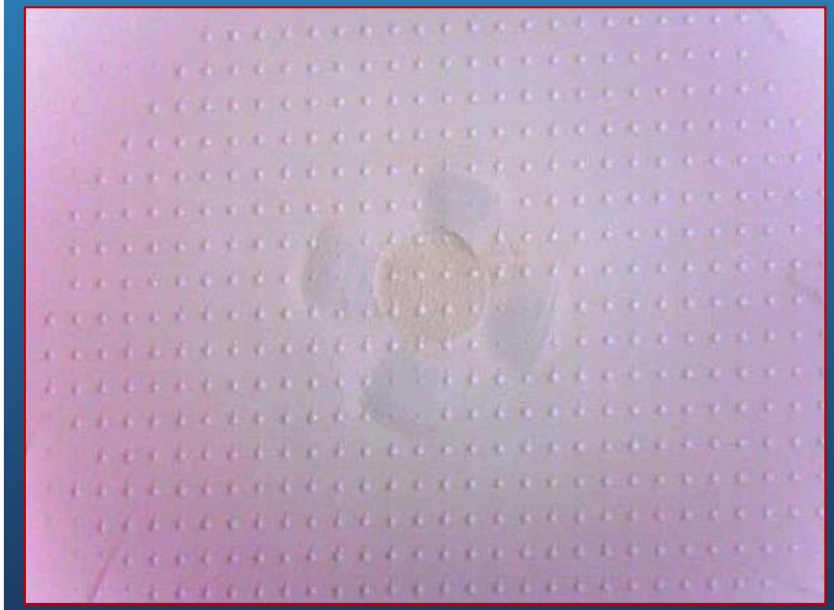


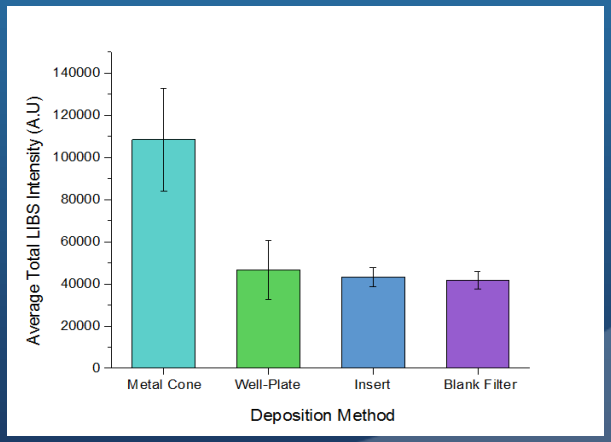
Concentration curve

limit of detection of 5530±872 CFU per ablation event

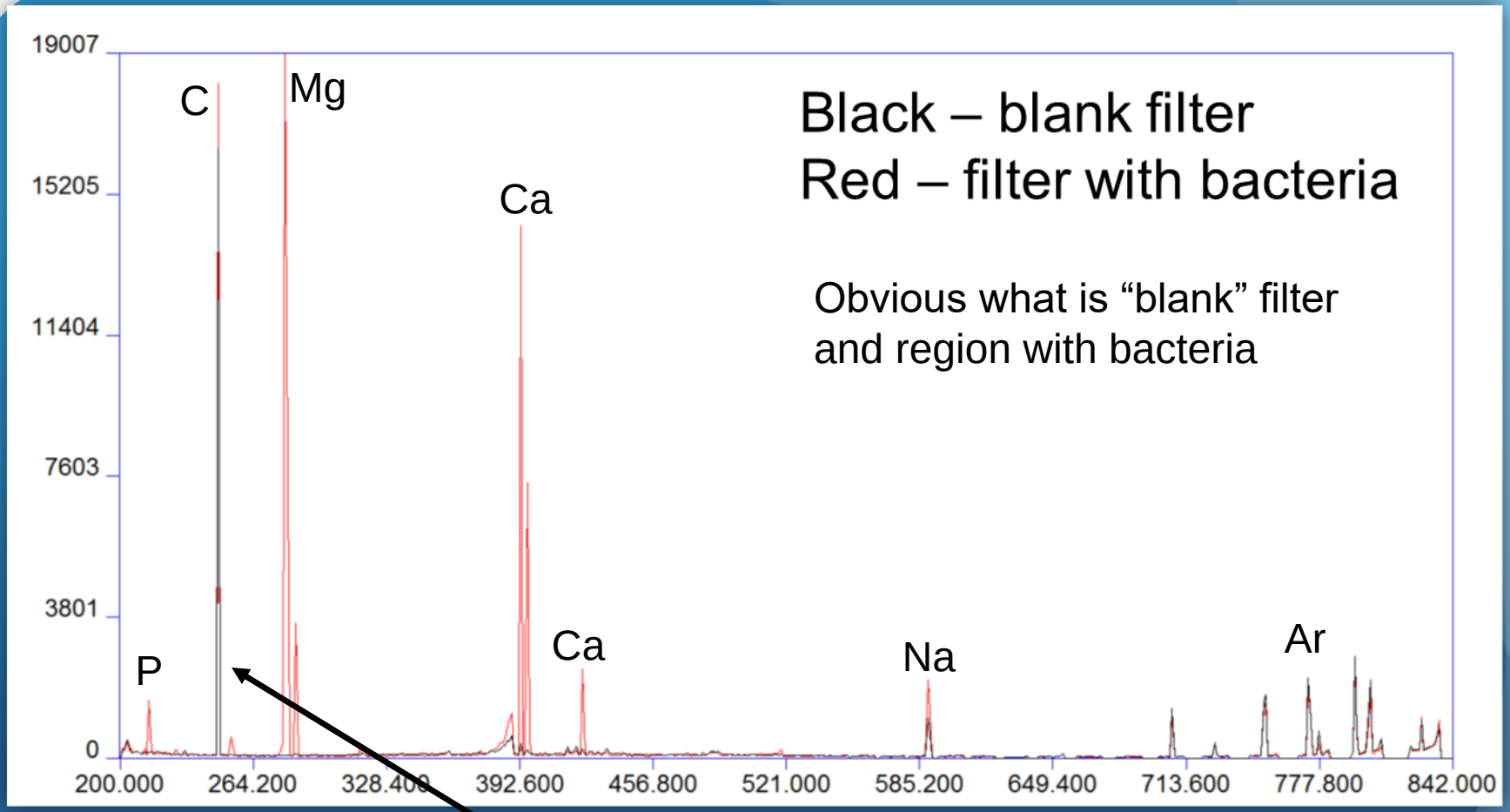


Linear region of concentration curve





Bacteria detected when other methods could not

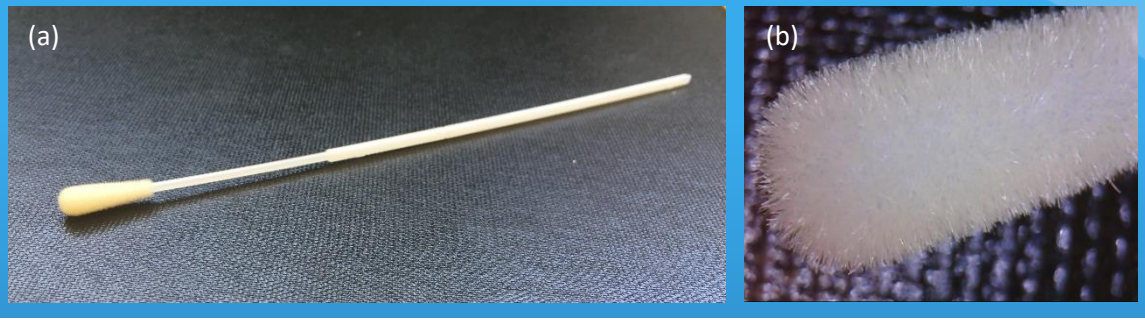


One of our biggest challenges: the C247 line dominates the spectrum at low / no concentration.

This large constant intensity limits the amplification we can use on the ICCD before damage.

Suggestions?

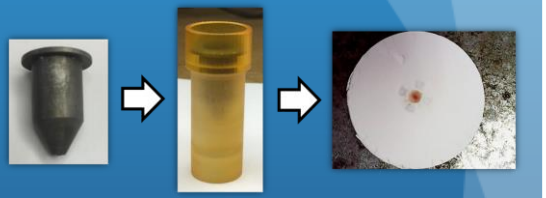
New Collection Procedure: Swabs

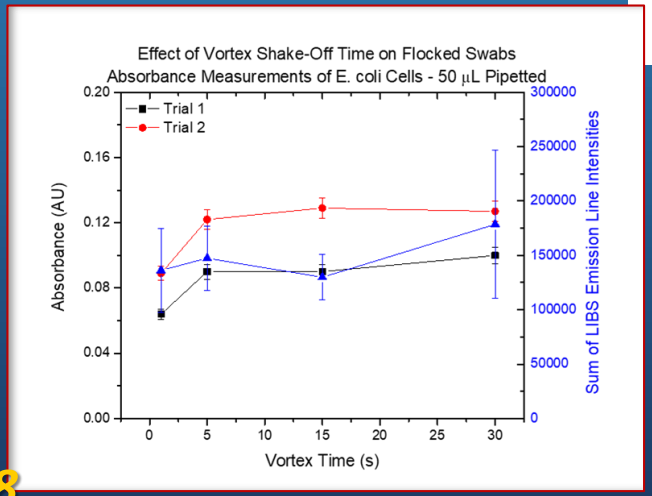


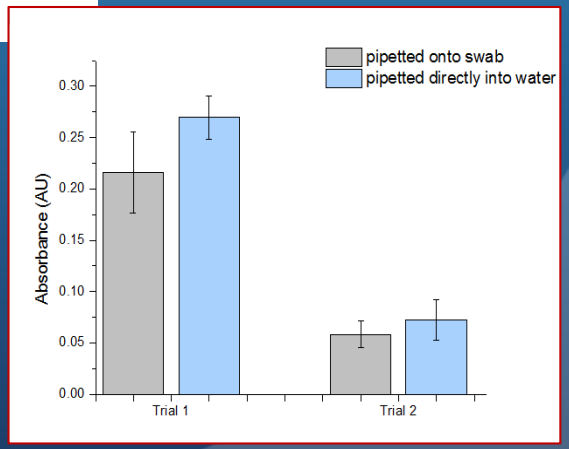
(a) Flocked swab used in this work. (b) Flocked swab zoomed-in on the tip

Cannot shoot right on the swab

- Far too irregular (almost no plasma)
- Cells not concentrated

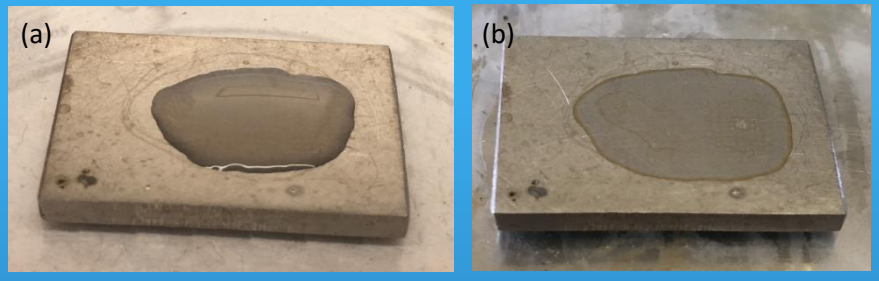






2017-2018

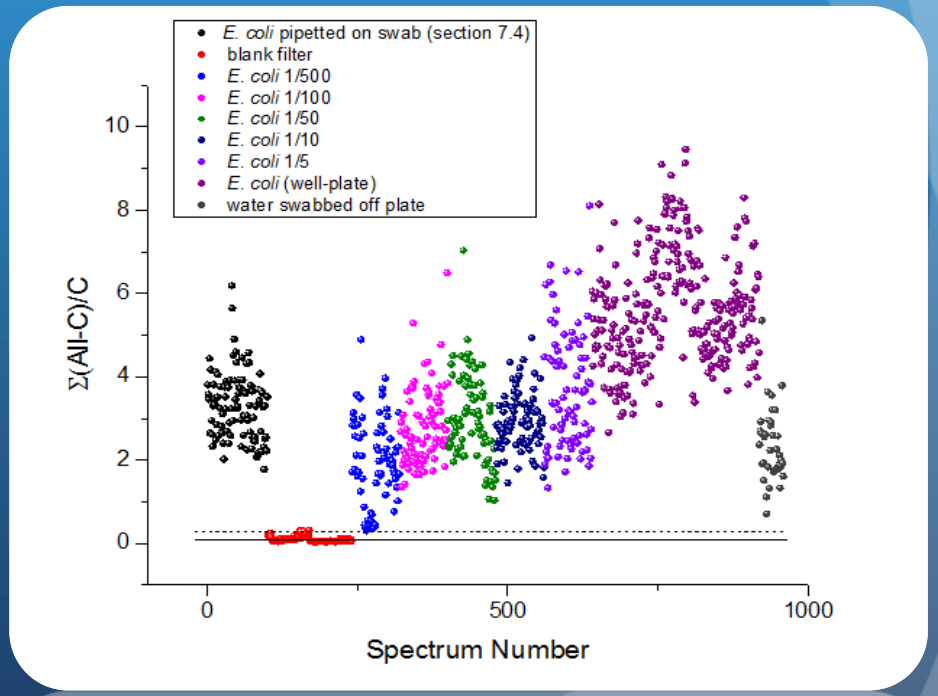
New Collection Procedure: Swabs



(a) 100 μL of E. coli pipetted onto surface of metal plate. (b) Metal plate after heated on hot-plate for 2 minutes 20 seconds at 200 °C. Water has evaporated and film of bacteria is observed

Dilution	Initial absorbance (AU)	Final absorbance (AU)			
		1	2	3	4
1/5	2.486	0.224	0.131	0.267	0.254
1/10	2.056	0.137	0.159	0.178	0.177
1/50	0.459	0.015	-0.007	-0.008	-0.004
1/100	0.269	-0.015	-0.015	-0.012	-0.006
1/500	0.023	-0.027	0.006	-0.022	-0.015

It was found that for the 1/5 dilution, approximately 88% of the bacteria that were deposited on the metal plate were picked up by the swab and released in water, and for the 1/10 dilution, approximately 79% were picked up and released in water.



What's Next

- Lowering LOD by eliminating C247 "contamination."
- Continue reducing titer.

1. Try different types of bacteria (cocci versus rods).
2. Continue experiments with swabs. Reduce spectral contribution from non-bacteria.
3. Culture bacteria in liquid media. Further study use of Tween 20 to prevent sticky clumping of cells which should improve repeatability and increase transfer efficiency.

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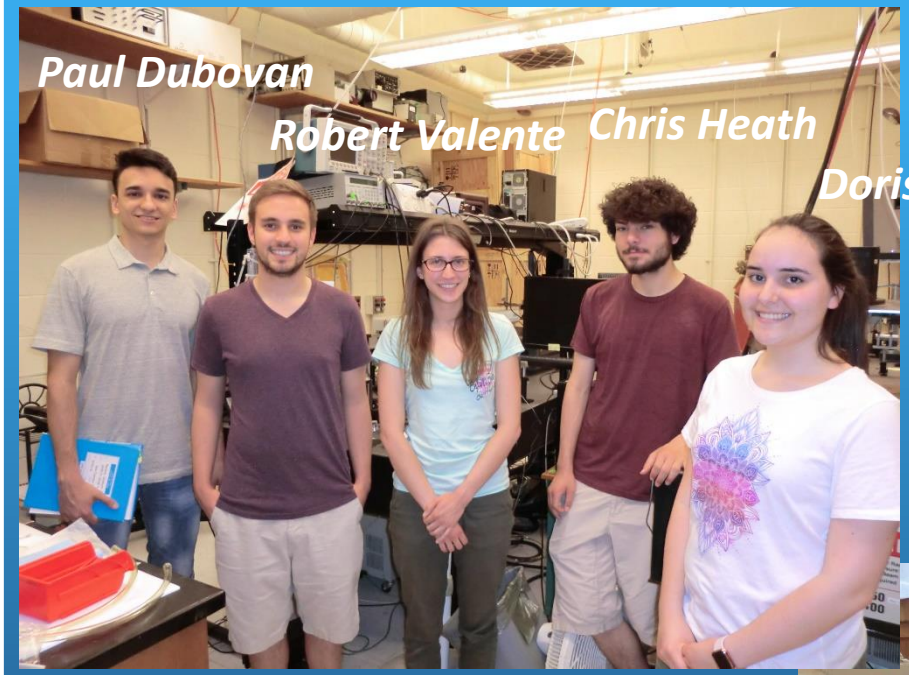
- *University of Windsor* Faculty of Science

All Credit to the Students!



Allie Paulick