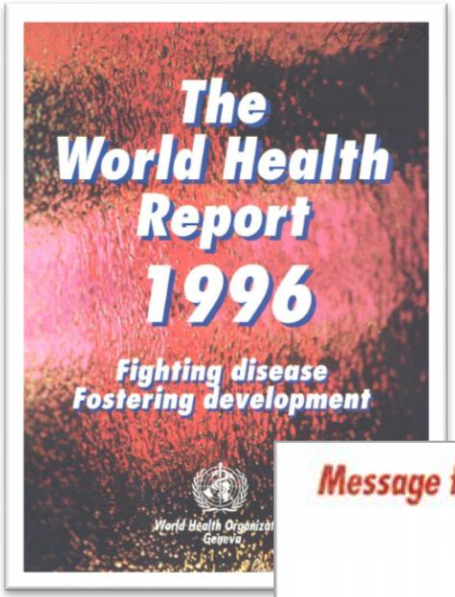
Development of Laser-Induced Breakdown Spectroscopy as a Rapid Diagnostic Tool for Bacterial Infection

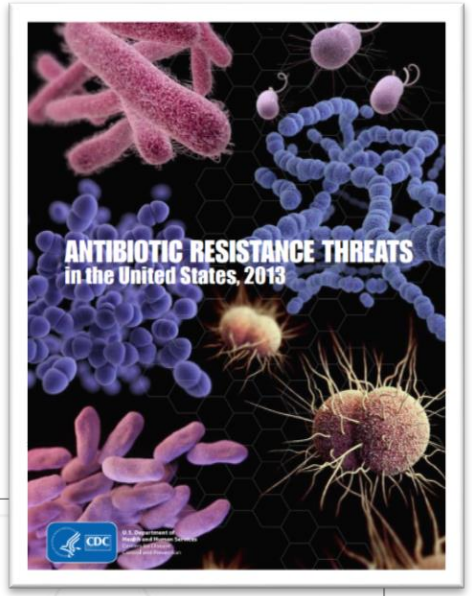
Alexandra Paulick
Department of Physics
University of Windsor

Outline

- Motivation
- Laser-Induced Breakdown Spectroscopy
- Methodology
- Apparatus
- Experimental Results
  - Technique to separate unwanted material from bacterial suspension
  - New sample preparation procedure
  - Bacteria collected with swabs
- Conclusions
- Future Work

Motivation





THE THREAT OF ANTIBIOTIC RESISTANCE

Introduction

Antibiotic resistance is a worldwide problem. New forms of antibiotic resistance can cross international boundaries and spread between continents with ease. Many forms of resistance spread with remarkable speed. World health leaders have described antibiotic-resistant microorganisms as "nightmare bacteria" that "pose a catastrophic threat" to people in every country in the world.

Each year in the United States, at least 2 million people acquire serious infections with bacteria that are resistant to one or more of the antibiotics designed to treat those infections. At least 23,000 people die each year as a direct result of these antibiotic-resistant infections. Many more die from other conditions that were complicated by an antibiotic-resistant infection.

In addition, almost 250,000 people each year require hospital care for *Clostridium difficile (C. difficile)* infections. In most of these infections, the use of antibiotics was a major contributing factor leading to the illness. At least 14,000 people die each year in the United States from *C. difficile* infections. Many of these infections could have been prevented.

Antibiotic-resistant infections add considerable and avoidable costs to the already overburdened U.S. healthcare system. In most cases, antibiotic-resistant infections require prolonged and/or costlier treatments, extend hospital stays, necessitate additional doctor visits and healthcare use, and result in greater disability and death compared with infections that are easily treatable with antibiotics. The total economic cost of antibiotic resistance to the U.S. economy has been difficult to calculate. Estimates vary but have ranged as high as $20 billion in excess direct healthcare costs, with additional costs to society for lost productivity as high as $35 billion a year (2008 dollars).[1]

- Current methods of bacterial identification in a clinical setting
  - require transferring the sample to a lab
  - require expertise in microbiology
  - expensive/labor-intensive
  - may only be useful for certain types of bacteria
  - **slow**

  For example: standard culturing techniques for bacterial identification take **1-3 days**

- Patients are treated with broad-spectrum drugs that have given rise to the crisis of antibiotic resistant bacteria

- Rapid and accurate diagnosis of bacterial infection are required so that **more targeted treatment can begin as soon as possible**

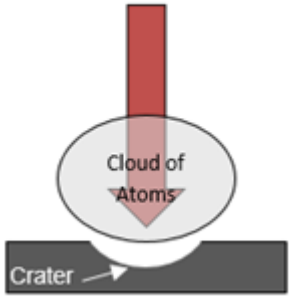
- Laser-induced breakdown spectroscopy (LIBS)
  - identify bacteria in research settings
  - has potential to detect and identify bacteria in clinical specimens (i.e. blood/urine samples, throat/nasal swabs, etc.) ***within minutes***

- **Goal:**
  LIBS as a **rapid point-of-care diagnostic tool** in a clinical setting
    - This includes developing **quick bacterial preparation methods** prior to testing that utilizes equipment and methods that are **common or easy to implement in a clinical setting**

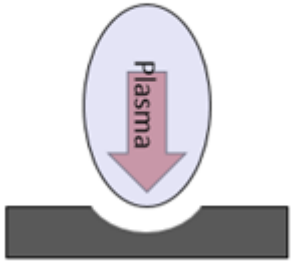
Laser-Induced Breakdown Spectroscopy (LIBS)

LIBS is an **elemental analysis technique**

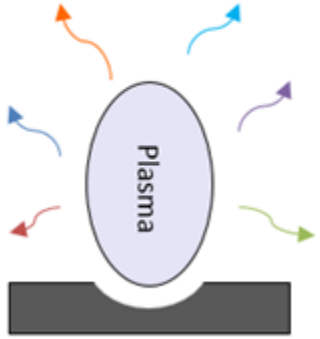
Pulsed laser is focused on target surface which absorbs laser energy



Target material is **vaporized**, generating a cloud of atoms above the target surface



Cloud of atoms absorbs the remaining laser energy, forming a **plasma**



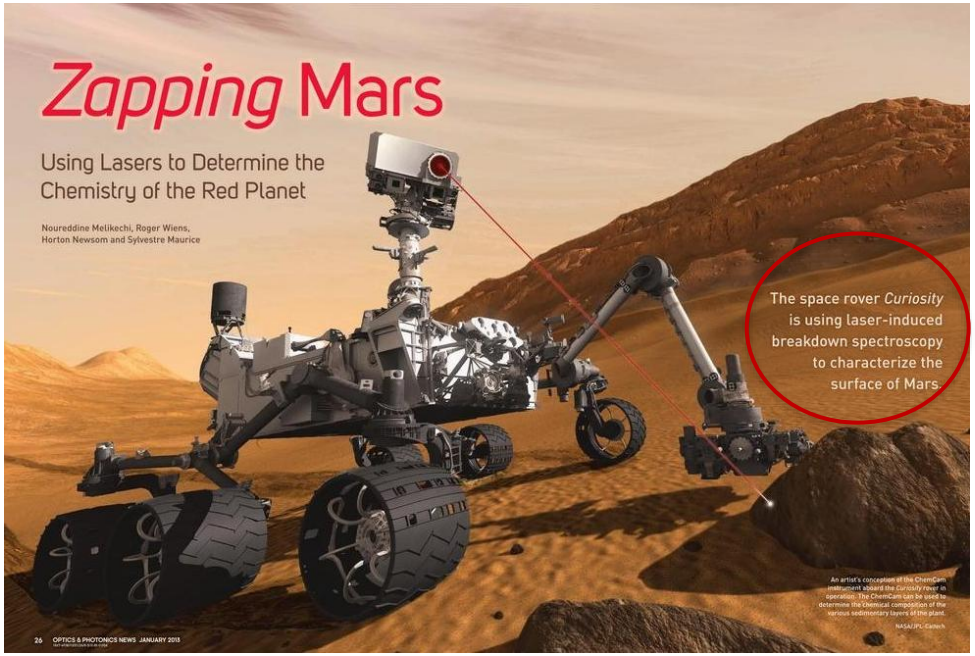
As the plasma cools, photons are emitted and collected for elemental analysis

LIBS Advantages

- Can be done on **solids**, **liquids**, **gases** and **bacteria**
- Little to no sample preparation
- Requires only **μg** of sample (can detect *attograms* of an element)
- **Fast:** elemental composition can be determined in under *1 second*
- Simultaneously detects **all elements in periodic table**
- The use of the laser allows for **point sampling** & **elemental mapping**

- **Benchtop** and **portable hand-held** LIBS devices have been made

- LIBS can be done **remotely**



Previous Results for LIBS on Bacterial Samples

- Discriminate between **bacteria** and other **biotypes** (molds, pollens)

Bacteria are discriminated based on elemental composition

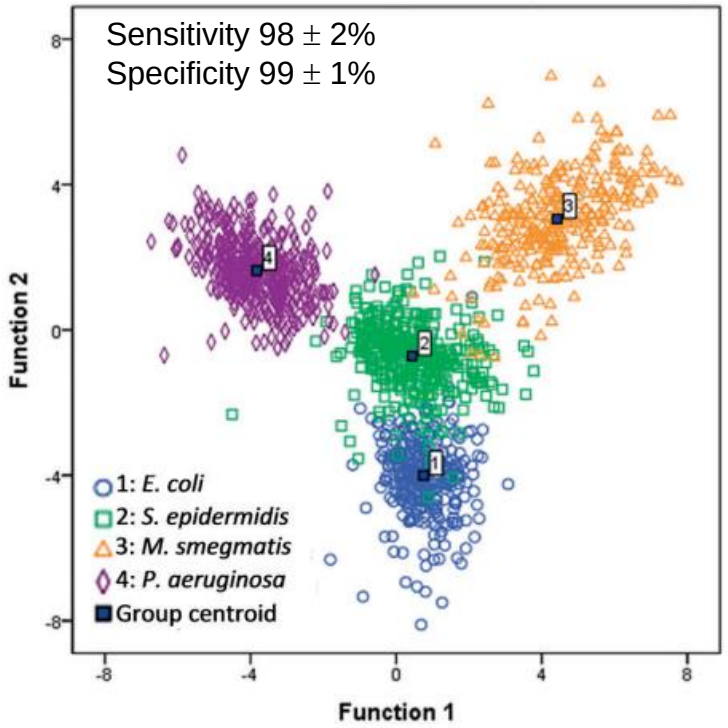
Bacterial spectra are classified using discriminant function analysis (**DFA**)

- Unknown spectra are classified against a precompiled library of known spectra

Bacterial library:

- 164 independent variables (intensities of elemental lines and ratios of these lines to each other)
- ~ 1500 spectra acquired over 3 months from 4 species of bacteria (*E. coli, S. epidermidis, M. smegmatis, P. aeruginosa)*

Bacterial classification based on elemental composition measured by LIBS

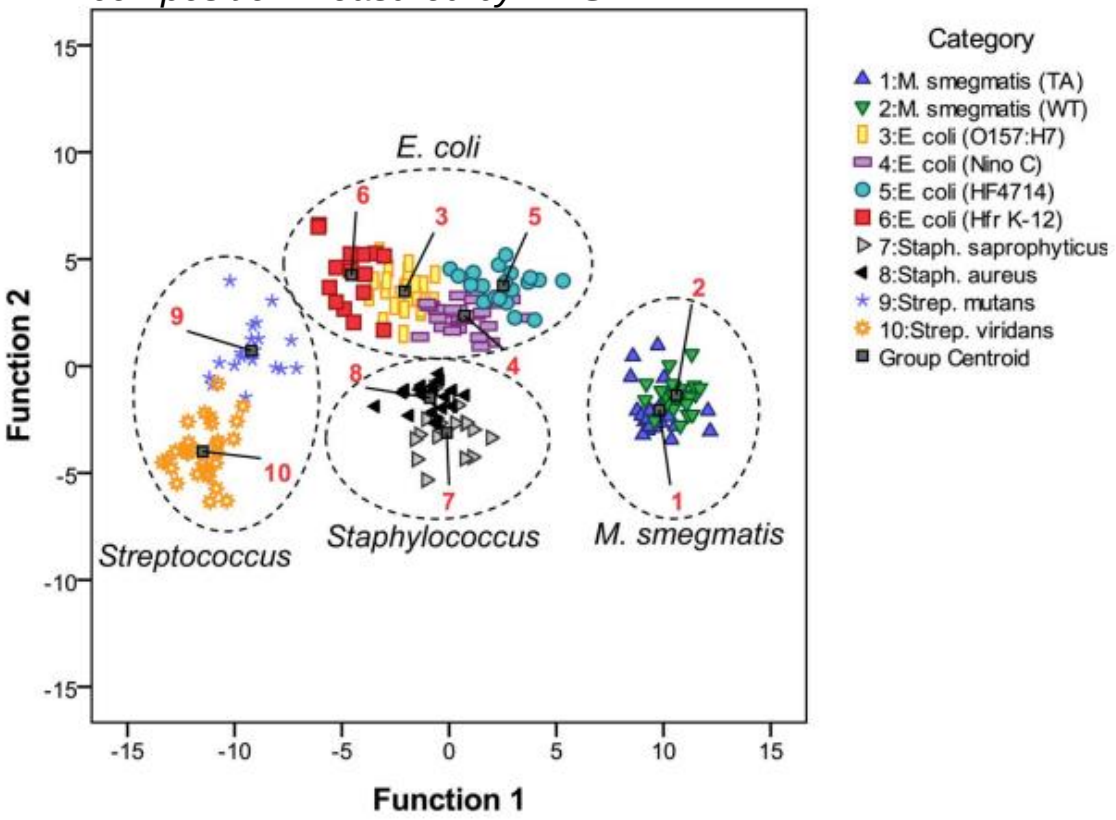


D. J. Malenfant *et al.*, Appl. Spectrosc., **70** (3), 485 (2016)

Genus	Species	Strain
Escherichia	*coli*	O157:H7
Escherichia	*coli*	K-12
Streptococcus	*mutans*	
Streptococcus	*viridans*	

- Discriminate between different **strains** of a single species of bacteria
- LIBS spectrum of a certain bacterial species **does not change over time**
- LIBS spectra from different species and strains **naturally group together** according to **genus**

Bacterial classification based on elemental composition measured by LIBS



S. J. Rehse *et al.*, Appl. Opt., **49** (13), C27 (2010)

- Bacterial identification is **independent of growth conditions** of bacteria
- **Membrane biochemistry** of bacteria contributes to the LIBS-based identification
- Majority species in a two-species mixture can be identified provided it comprises **at least 70%** of the mixture
- LIBS determined to be feasible for **diagnosing UTI's**

Limitations of Previous Results

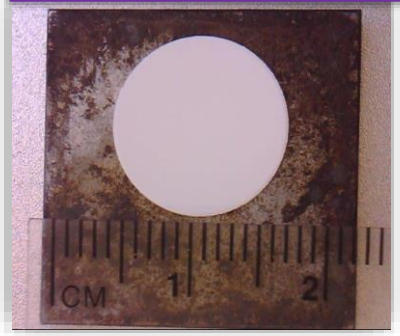
- Showed that LIBS is capable of bacterial identification in **idealized lab settings**
- Much of this work involved **proof-of-concept** experiments and has not yet fully addressed all aspects of **actual clinical specimens**
- Things to consider for actual clinical specimens:
  - Unwanted material mixed in with bacteria (i.e. red/white blood cells in blood)
  - Amount of bacteria present in a specimen
  - Nature of the sample collection procedure (some samples collected with swabs)
  - Easy and inexpensive sample preparation methods

Overview of Methodology

1 Bacteria is cultured on TSA plates

2 Bacterial cells are removed and suspended in 1.5 mL deionized water

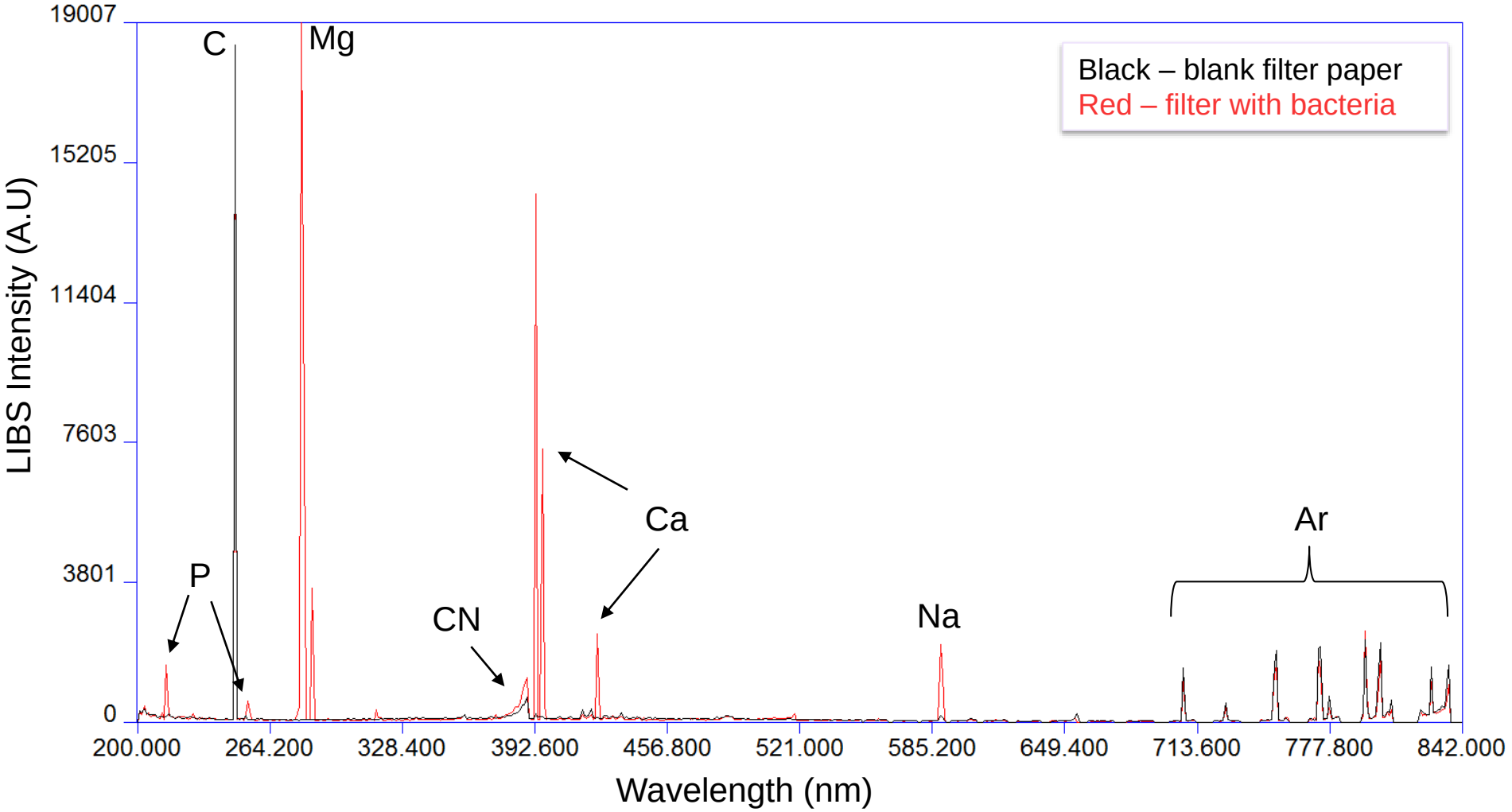
3 Bacterial suspension is vortexed and deposited on nitrocellulose filter paper



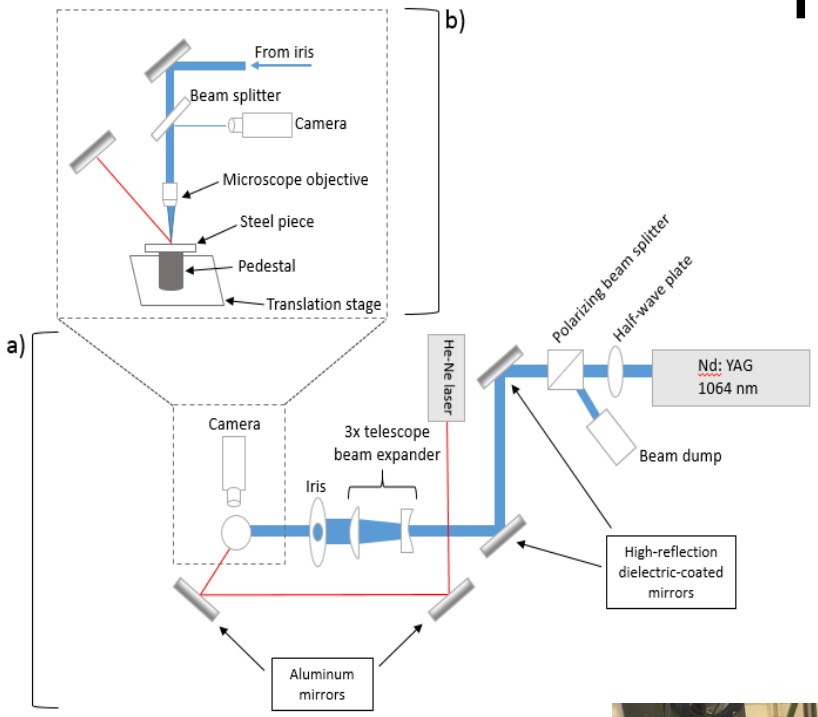
4 Filter paper is mounted on a steel piece and ablated with laser

5

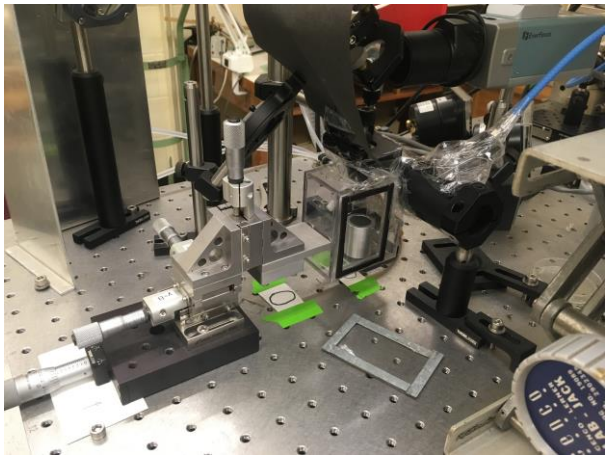
After laser ablation, light from the plasma is dispersed, revealing the sample's elemental composition

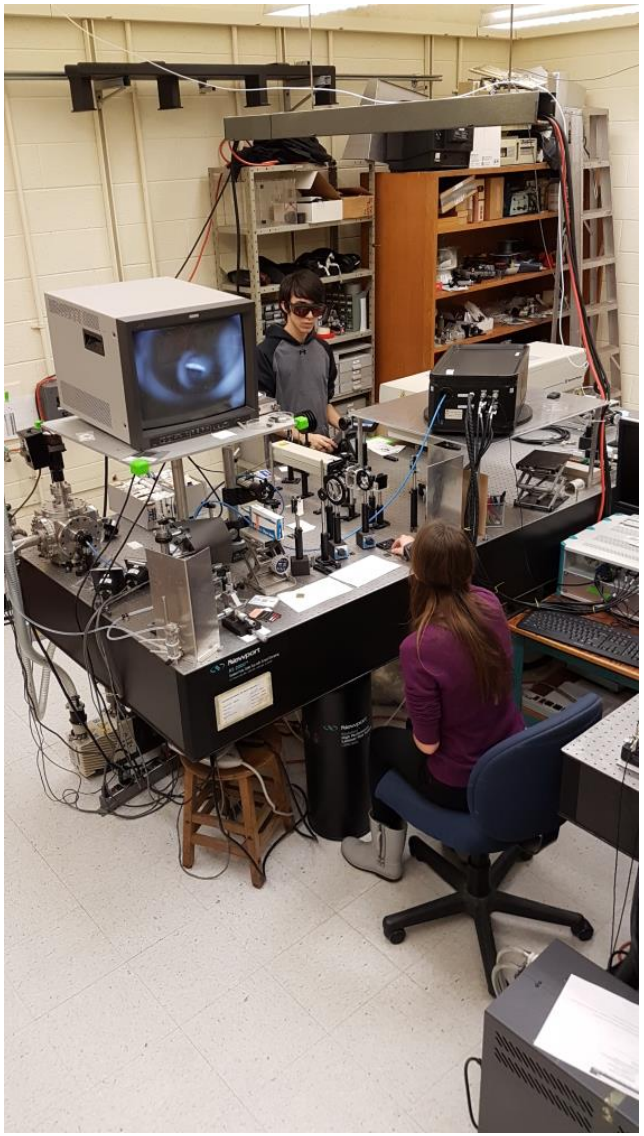


Apparatus



(a) Overhead schematic of the optical train used to direct laser pulses to the target. (b) Schematic side view of laser pulses emerging from the iris and directed to a target which is mounted on a steel piece



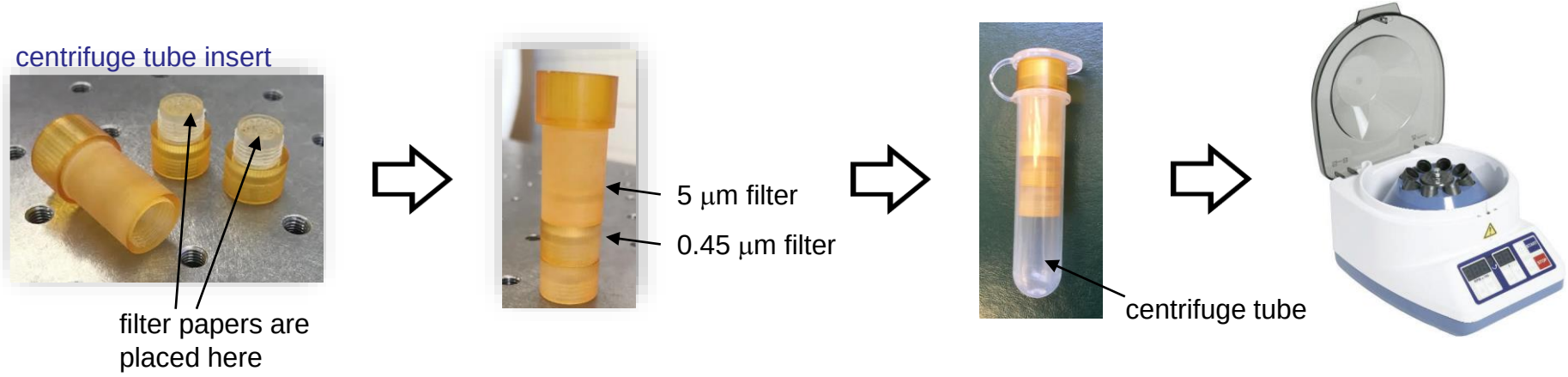


Preparation Method to Separate a Contaminant from a Bacterial Suspension

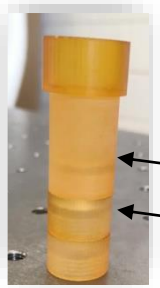
Biological samples (blood sample, swab sample, etc.) will likely contain **unwanted cells** that would need to be separated from the bacteria before testing with LIBS

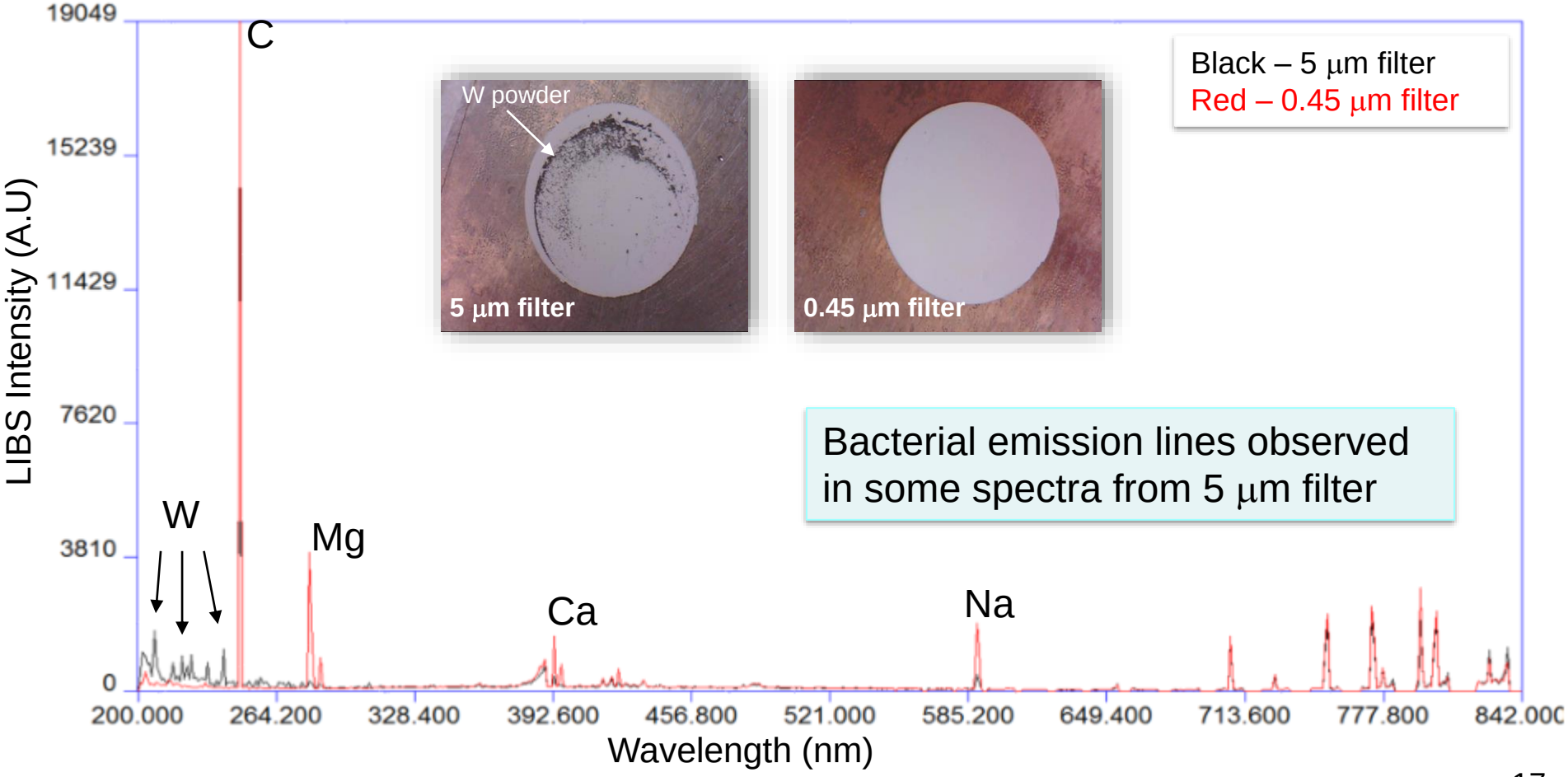
Cell sizes:
- Bacteria ~ 1 μm
- Red blood cell ~ 6 – 8 μm
- Eukaryotic cells ~ 10 – 100 μm

Isolate the bacteria using filter papers with **different pore sizes** (5 μm and 0.45 μm)

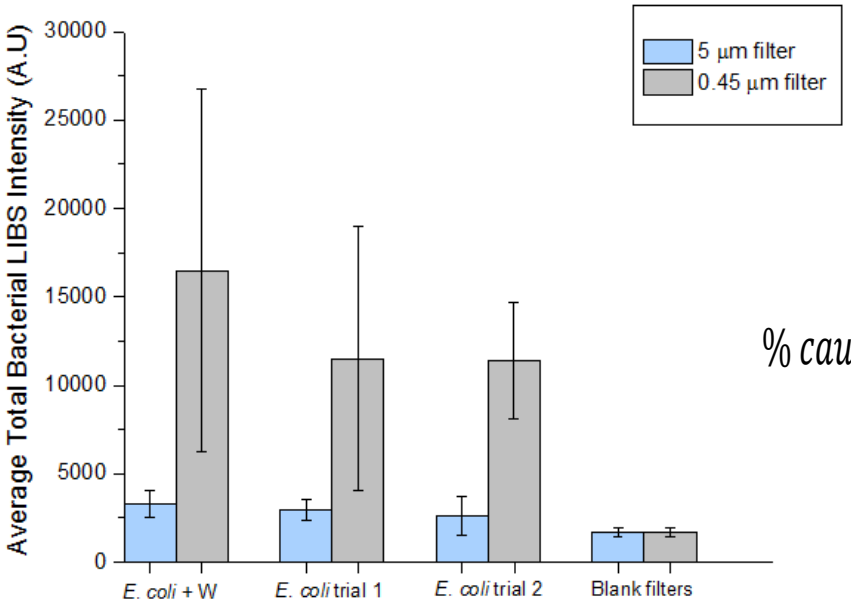


Tungsten powder (12 μm average particle size) added to *E. coli* suspension to simulate unwanted cells in a biological sample





How much bacteria is caught on the 5 μm filter?

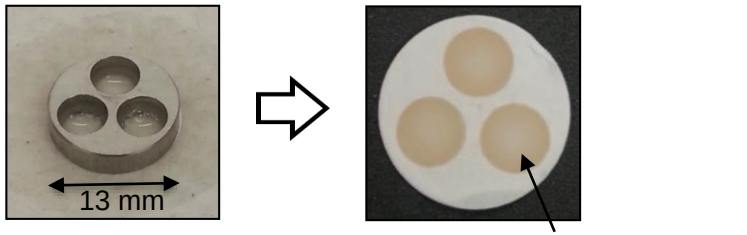


$$\%\ caught\ on\ 5\mu m\ filter = \frac{\left(I_{5\mu m} - I_{5\mu m}^{blank}\right)}{\left[\left(I_{5\mu m} - I_{5\mu m}^{blank}\right) + \left(I_{0.45\mu m} - I_{0.45\mu m}^{blank}\right)\right]} * 100\%$$

~ 10% caught on 5 μm filter → ~ 90% pass through

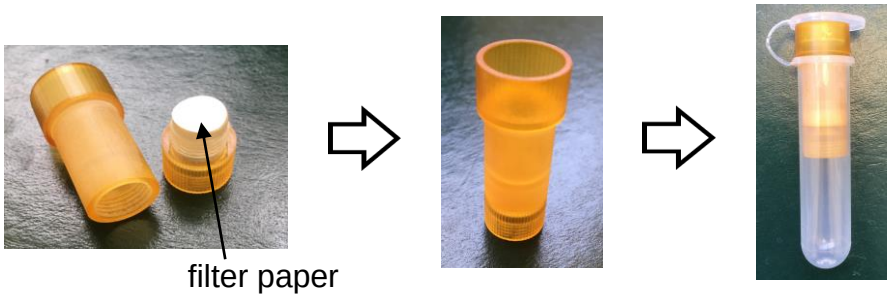
Previous Bacterial Deposition Procedures

1) Well-plate



Bacterial LOD **~ 50 000 CFU** per laser ablation event

2) Centrifuge tube insert



Bacterial LOD **~ 90 000 CFU** per laser ablation event

Number of bacterial cells present in clinical samples:

- < 100 CFU/mL in blood
- 0-200 CFU in typical nasal swab

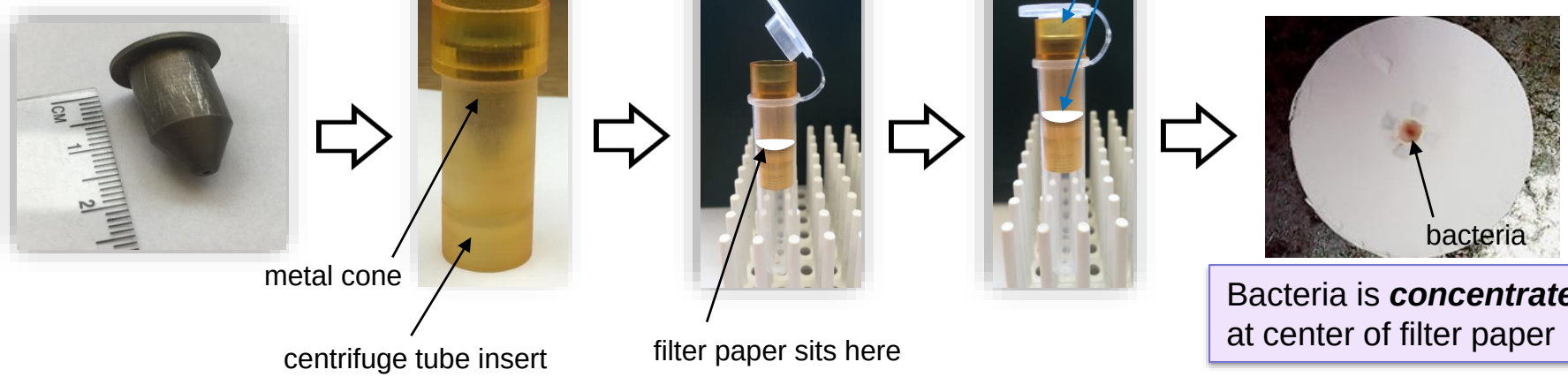
These LOD's are ***not clinically relevant***. Bacterial LOD with LIBS MUST be lowered.

New Deposition Procedure: Metal Cone

Metal cone to force deposition of bacteria onto *smaller region* at center of filter paper

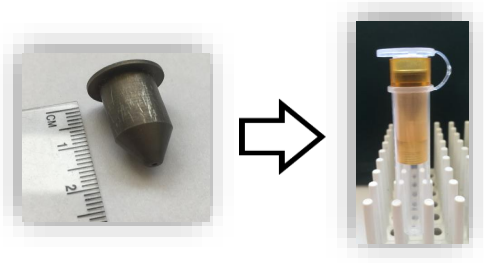
Why do this?
Increases the number of bacterial cells per unit area, leading to *more bacterial cells ablated in a laser shot* compared to previous deposition procedures

Method:

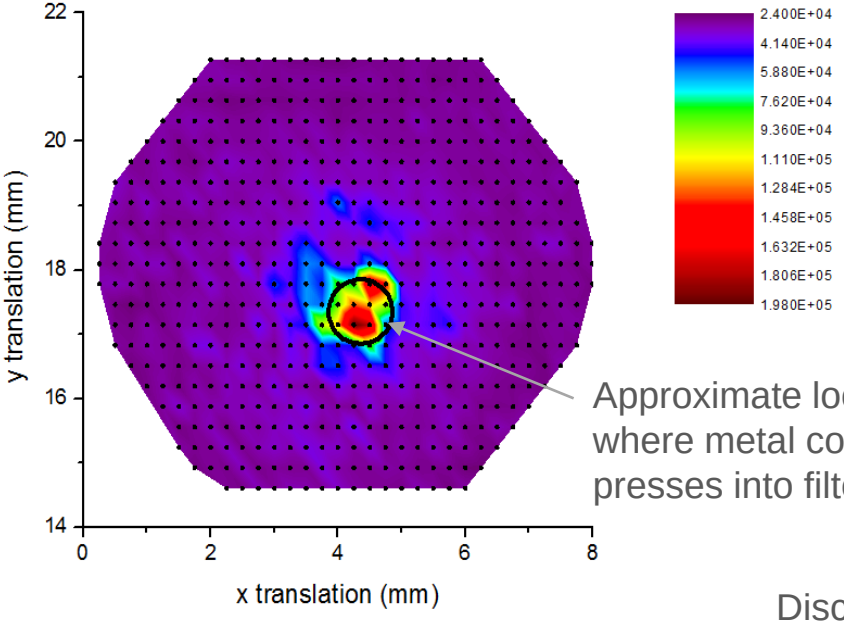


Metal Cone: Bacterial Concentration

- *E. coli* deposited on filter paper with metal cone
- 569 LIBS spectra acquired across filter



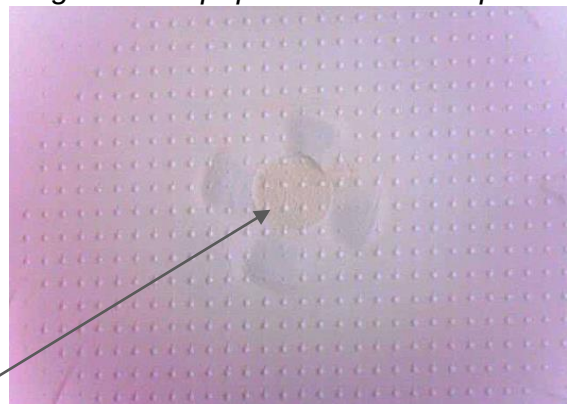
Intensity map depicting bacterial deposition on filter paper for bacteria deposited with metal cone



Approximate location of where metal cone presses into filter paper

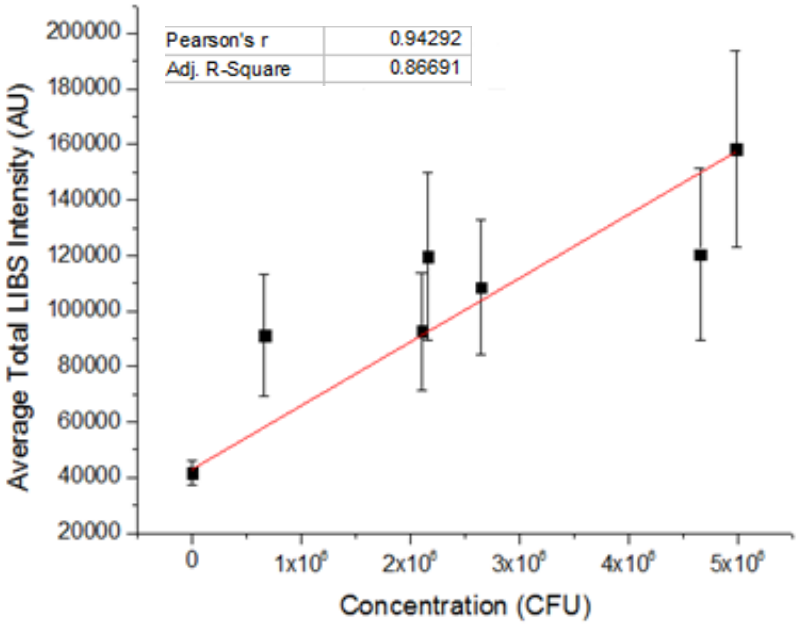
Colour indicates bacterial LIBS intensity
→ purple: no bacterial signal
→ red: strong bacterial signal

Image of filter paper after data acquisition



Discolouration due to presence of bacteria

Metal Cone: Limit of Detection



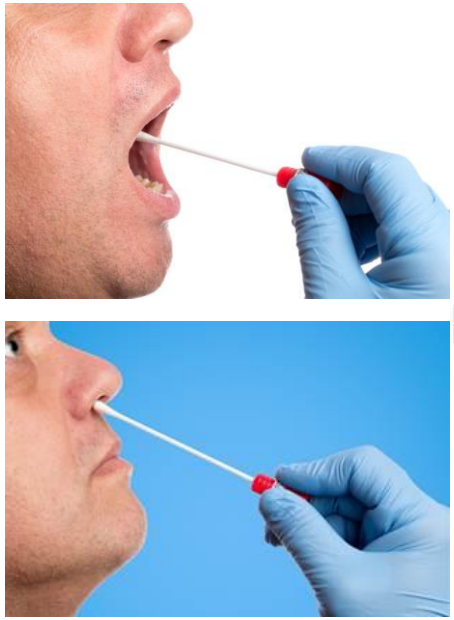
LOD ~ **5 500 CFU** per laser ablation event

metal cone **well-plate** **insert**

Recall:
Well-plate → LOD ~ 50 000 CFU per laser ablation event
Insert → LOD ~ 90 000 CFU per laser ablation event

Bacteria Collected with Swabs

Many clinical specimens are collected with swabs (throat, nose, ear, eye swabs)



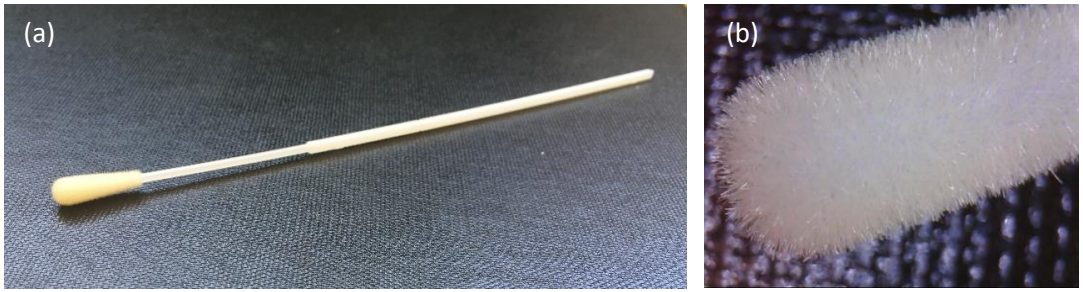
Swab is streaked on culture plate containing growth media for bacterial cells

If bacteria is present on the swab, it will grow on the plate

time consuming & require microbiology expertise

Can we use LIBS instead?

Want to ensure that samples collected using swabs can be tested with LIBS



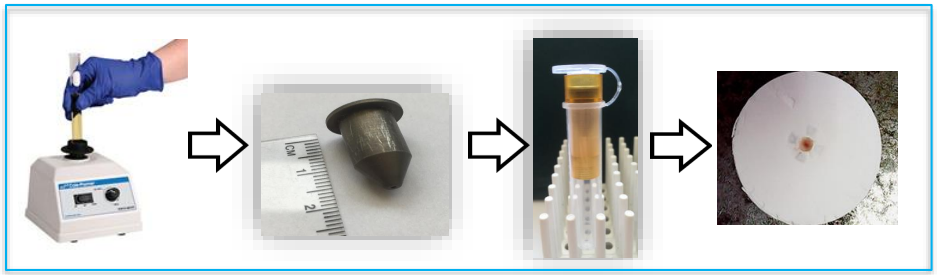
(a) Flocked swab used in this work. (b) Flocked swab zoomed-in on the tip

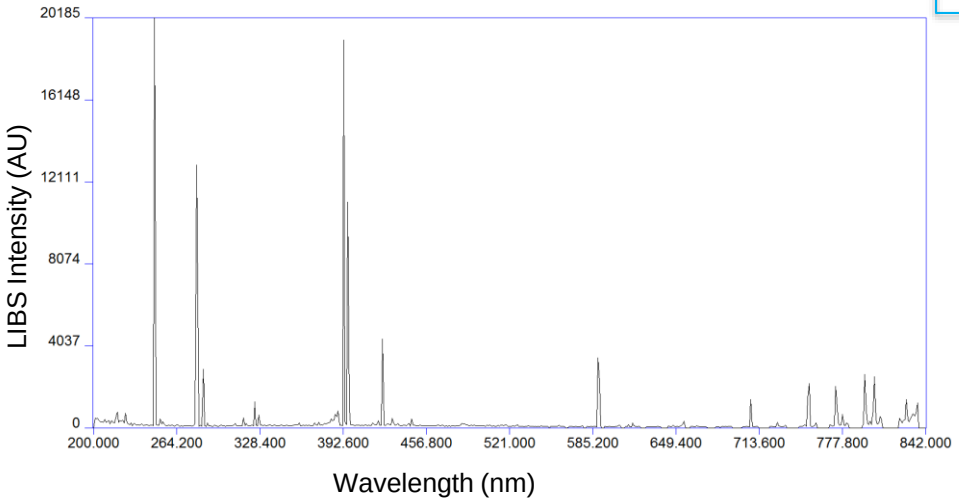
Cannot shoot right on the swab

→ Surface is too irregular

→ Bacterial cells are not concentrated

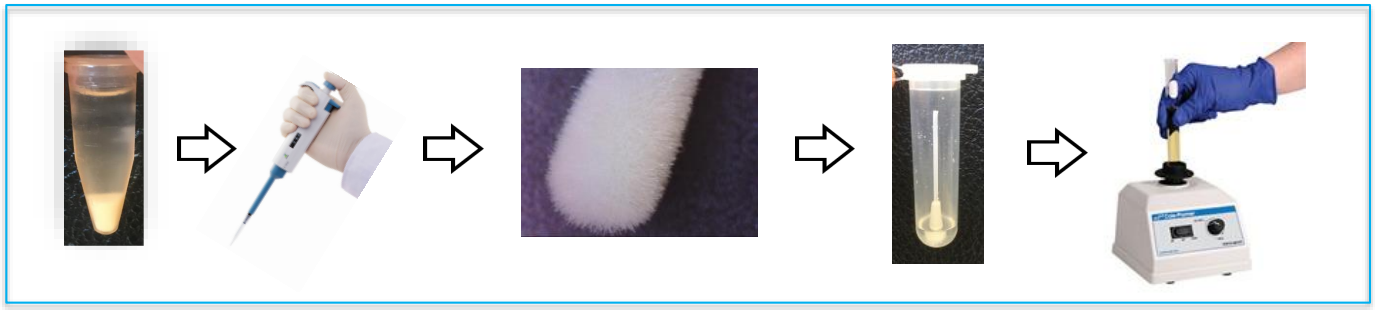
Instead of shooting swab ⇨

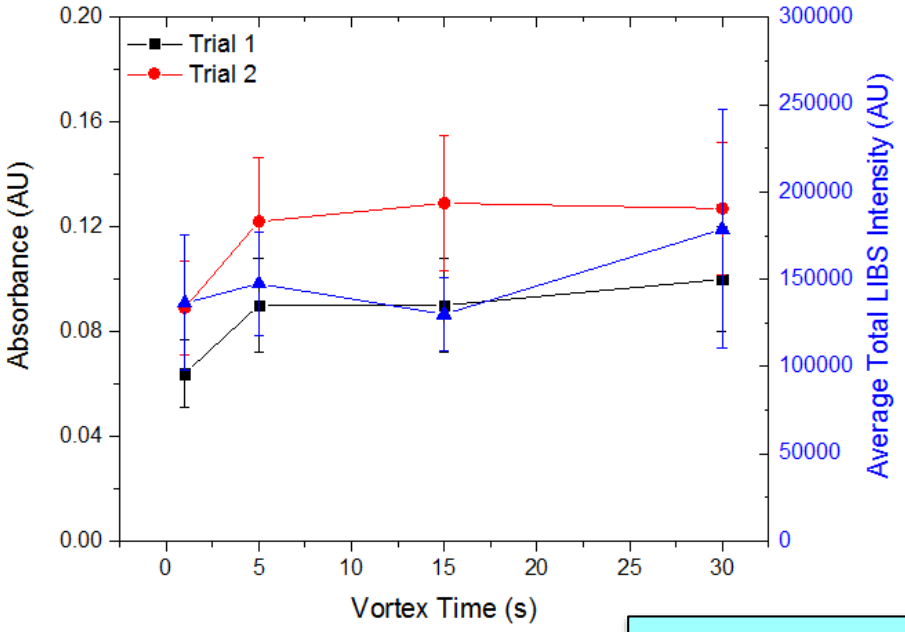




⇨ Capable of detecting bacteria shaken off a swab

How long to vortex the swab in water?



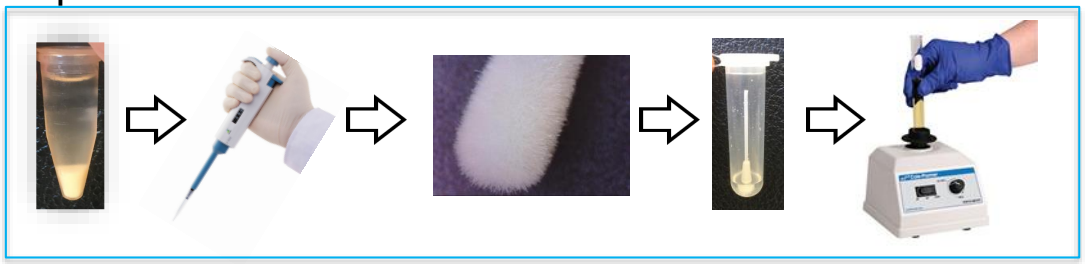


- All but the 1s vortex time released a similar amount of bacteria
- All vortex times exhibited the same average total LIBS intensity

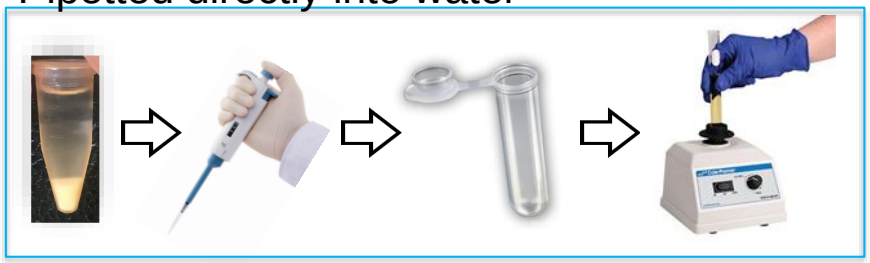
Recommended vortex time: **15 seconds**

How much bacteria is released from the swab by vortexing in water?

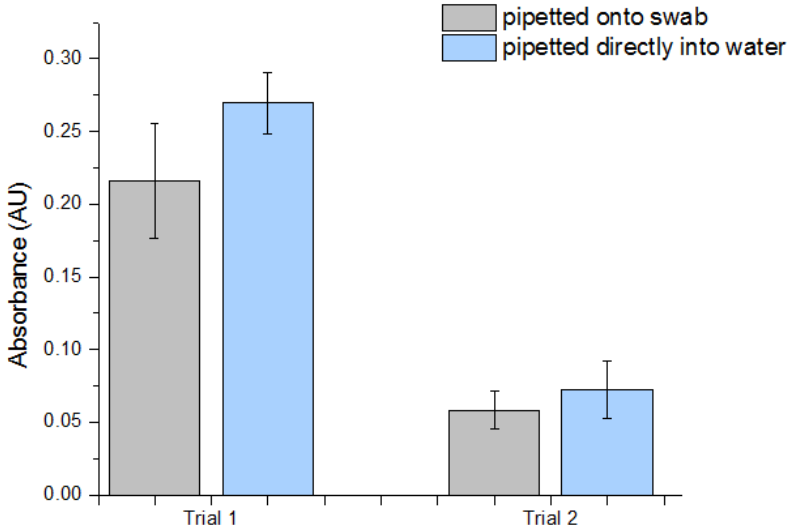
Pipetted onto swab



Pipetted directly into water



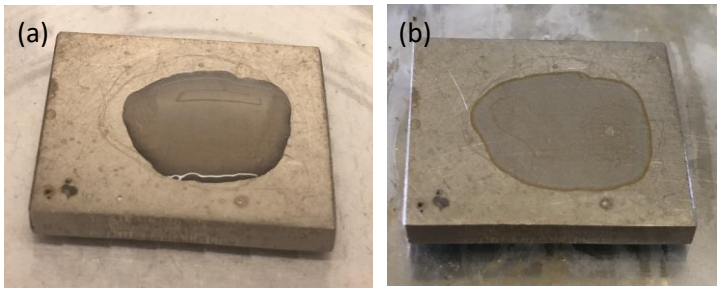
~ 80% of bacteria picked up by swab were released after vortexing in water



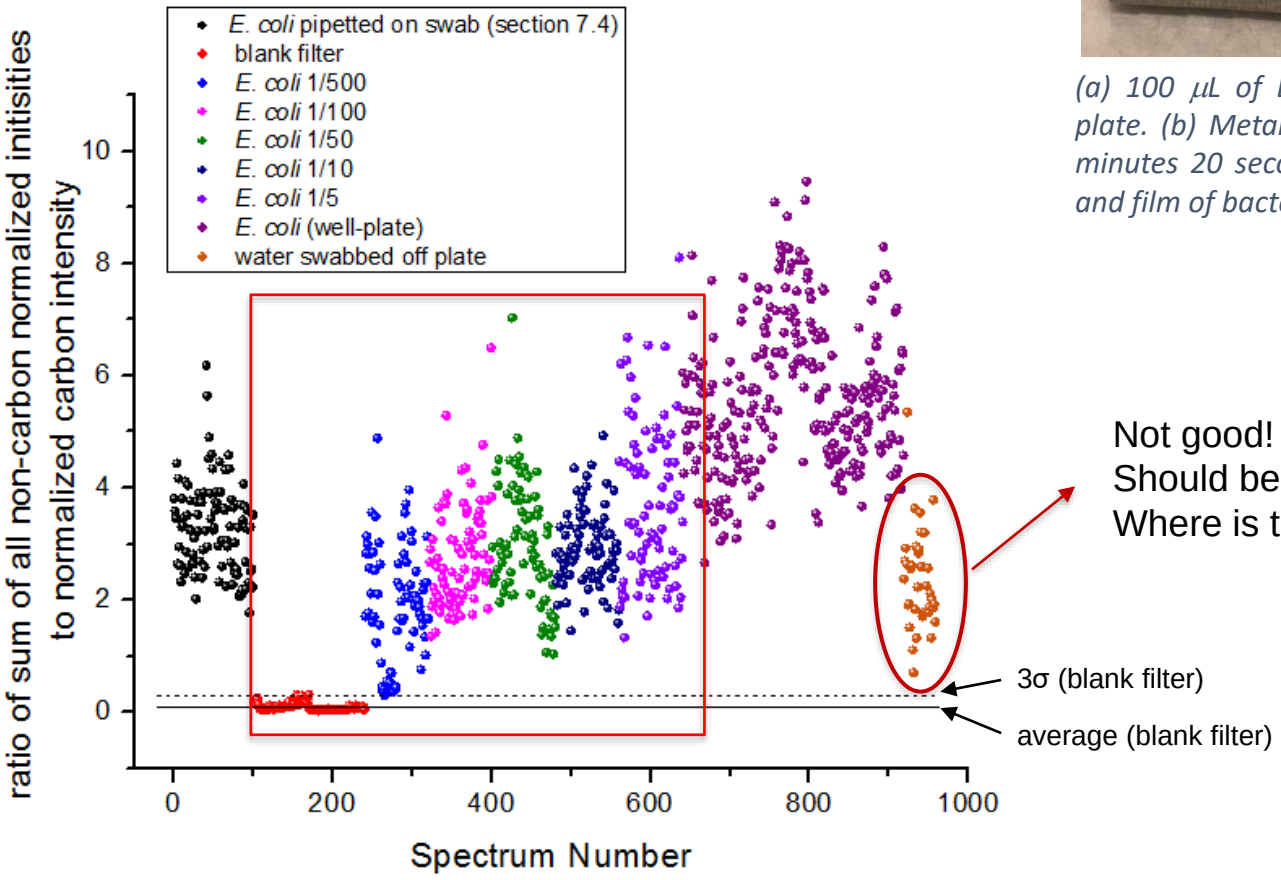
Average absorbance value plotted for samples prepared by pipetting a bacterial suspension onto a swab and vortexing it in water to release the cells and by pipetting directly into water. Error bars represent one standard deviation in the measurements

Can LIBS detect bacteria that have been swabbed off of a surface?

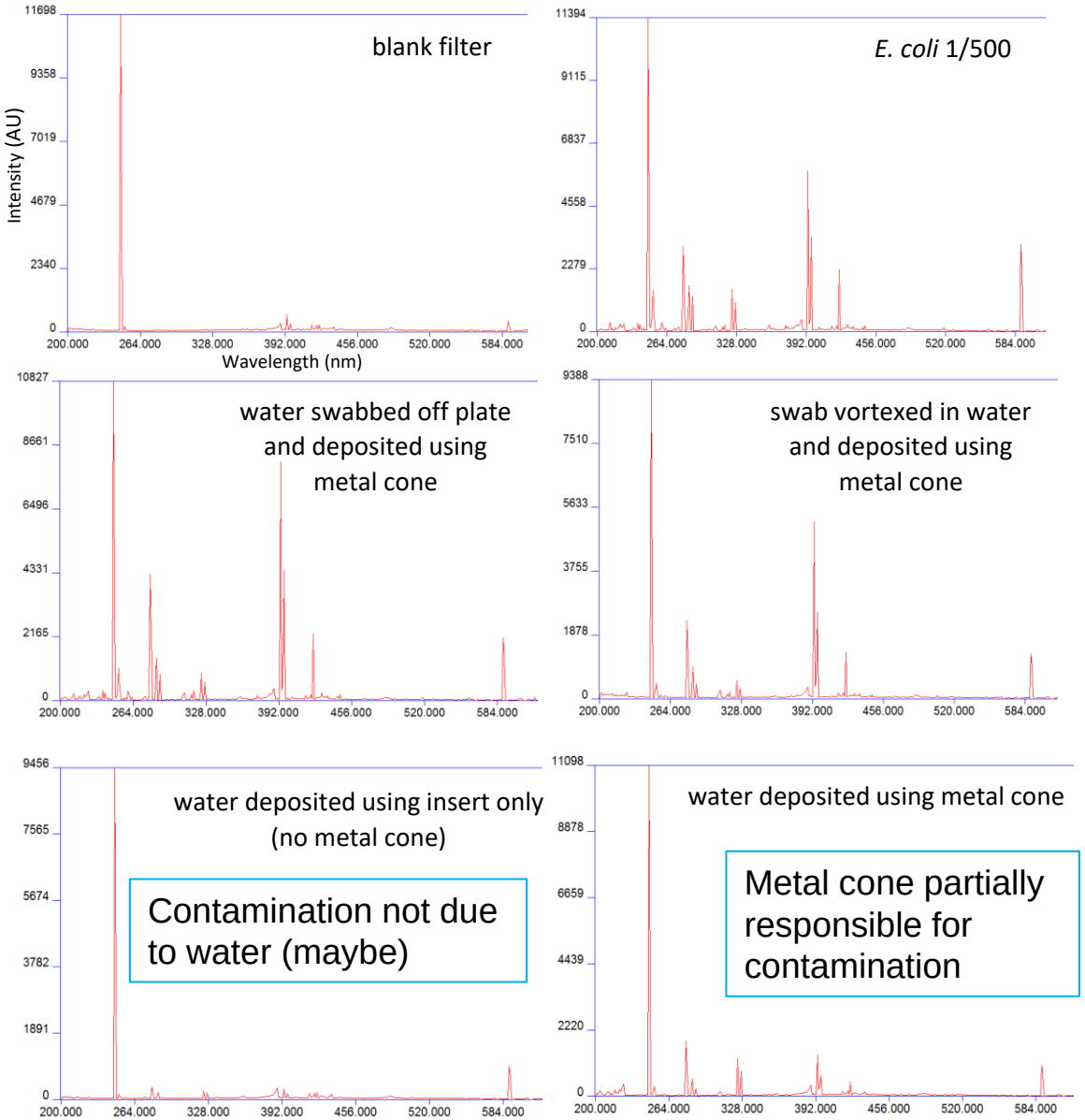
- More closely resembles specimen collection in a clinical setting
- Simulates hygiene surveillance tests in hospitals and food processing plants



(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



Not good!
Should be similar to blank filter.
Where is this contamination coming from?



Resulting averaged spectra from 20 single-shot LIBS measurements on different samples. All samples in this figure were tested at the same spectrometer amplification

- **Metal plate** may be largely responsible for the contamination
- Contamination should be **significantly reduced** after further investigation and implementation of a **sufficient cleaning method** for the metal plate and cone

Conclusions

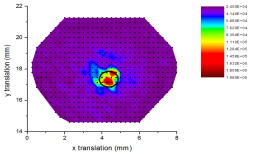
- Preparation method to separate unwanted material from bacterial suspension was effective

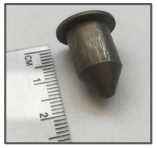
~ 10% of the bacteria are lost in this process

Future work:

- Reduce the amount of bacteria lost
- Test this method using a contaminant that more closely simulates biological cells, or begin testing on actual clinical specimens

- Metal cone:
  - effective at concentrating bacterial cells to a small region of the filter paper
  - lowered bacterial LOD by an **ORDER OF MAGNITUDE** compared to previous methods of bacterial deposition





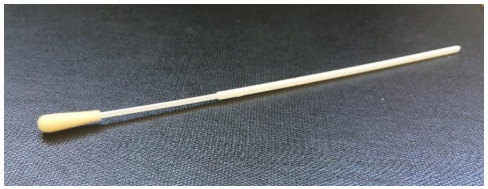
metal cone

well-plate

insert

Metal cone → LOD ~ **5 500** CFU per laser ablation event
Well-plate → LOD ~ **50 000** CFU per laser ablation event
Insert → LOD ~ **90 000** CFU per laser ablation event

- Bacteria collected with swabs:
  - Cannot shoot directly on swab
  - Optimal vortex time: 15 seconds
  - ~ 80% of the cells are released from the swab after vortexing
  - LIBS capable of detecting bacteria swabbed off a surface



Future Work:

- Identify and eliminate all sources of contamination
- Quantify bacterial identification (identification accuracy and LOD for bacteria swabbed off surface)

My Contributions to the Field

- Previous work in this field has shown that LIBS is capable of **rapidly identifying bacteria** in **ideal lab settings**

  Avoided the issues of **realistic sample preparation** by:

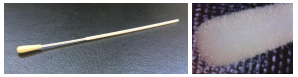
  1) Using pure cultures of bacteria
  2) Using unrealistically large concentrations of bacteria
  3) Not considering how clinical specimens are collected
  4) Using difficult, expensive, and/or time-consuming sample preparation methods

- Aim of this work was to address some of the issues related to the LIBS testing of **actual clinical specimens**

  1) Investigated a technique to separate bacteria from unwanted material

  2) Developed a new bacterial mounting procedure to reduce our LOD

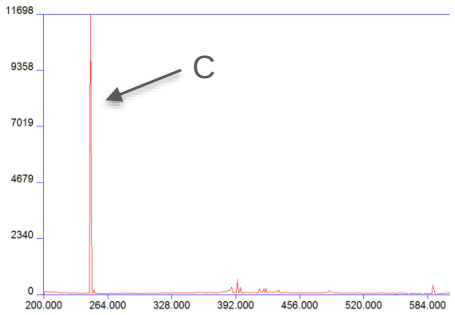
  3) Investigated whether LIBS could be used as a diagnostic tool for samples collected with swabs



  4) Used easy, inexpensive, and fast sample preparation methods
     - ➢ Easy to introduce to a clinical setting

Suggests that LIBS is a feasible diagnostic tool

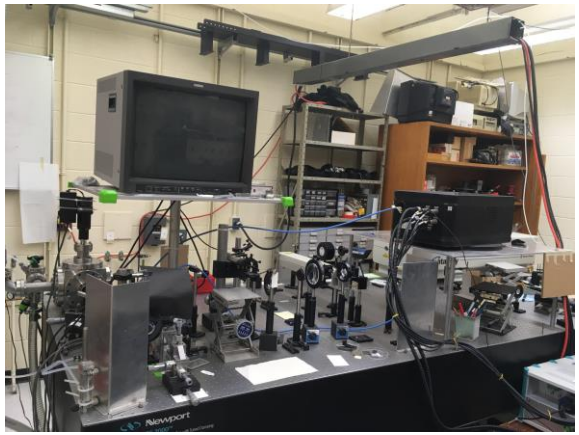
Future Work



- Reduce LOD
  - Mounting on different substrate (unlikely)
  - Eliminate detection from C
    - Notch filter to attenuate emission from C (expensive)
    - Multiple spectrometers with smaller wavelength coverage (expensive)

- Determine identification accuracy using chemometric techniques
  - How well can bacteria be classified when they are:
    - prepared using the methods developed in this work
    - obtained from different types of biological specimens (i.e. blood, CSF, urine, etc.)

- Optimize LIBS apparatus for clinical setting
  - Portable or benchtop LIBS device

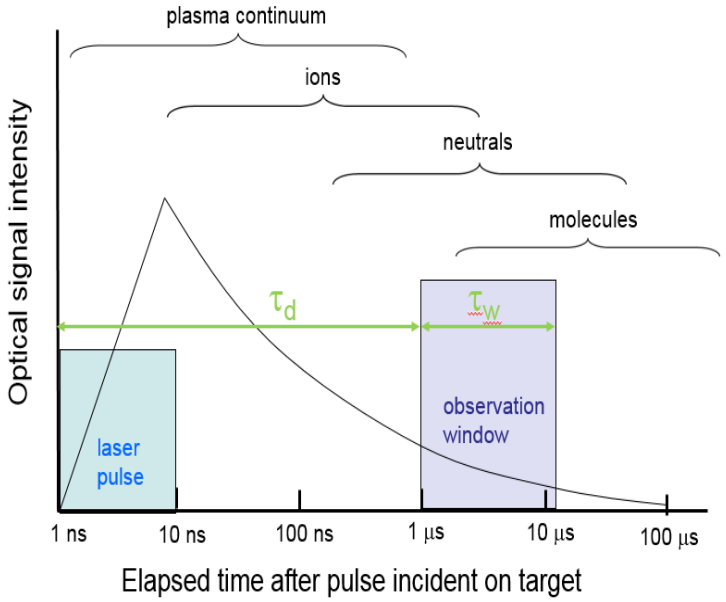


Acknowledgments

Thank you to:

- Dr. Rehse
- Mark Armstrong, Kevin Beaugrand, and Doris Rusu who prepared all of the swab samples
- Our research group and my friends and family

Element	Wavelength (nm)	Ionization State
C	247.856	I
P	213.618	I
P	214.914	I
P	253.398	I
P	253.560	I
P	255.326	I
P	255.491	I
Mg	279.079	II
Mg	279.553	II
Mg	279.806	II
Mg	280.271	II
Mg	277.983	I
Mg	285.213	I
Ca	317.933	II
Ca	393.366	II
Ca	396.847	II
Ca	422.673	I
Na	588.995	I
Na	589.593	I



Temporal evolution of a LIBS plasma

Emission lines
(i.e. C247 = 0, P213 = 1, …, Ca393 = 14, …, Na589 = 18)

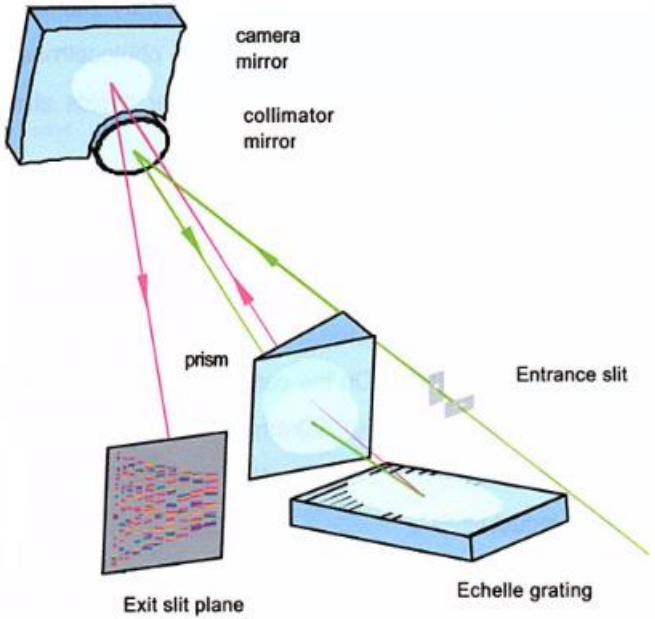
File name

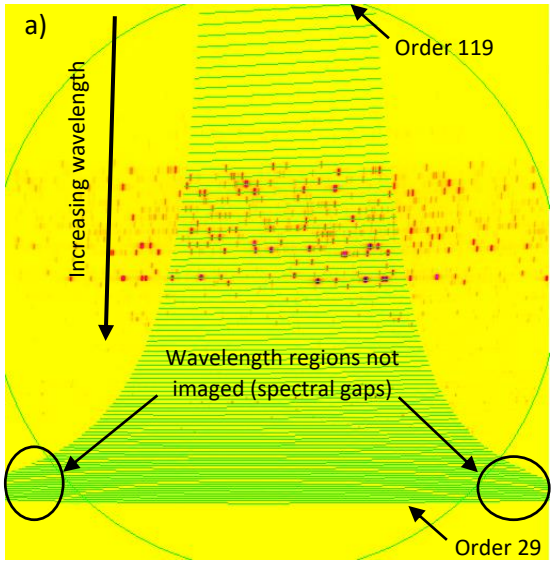
Emission intensity of C
(for 1st file)

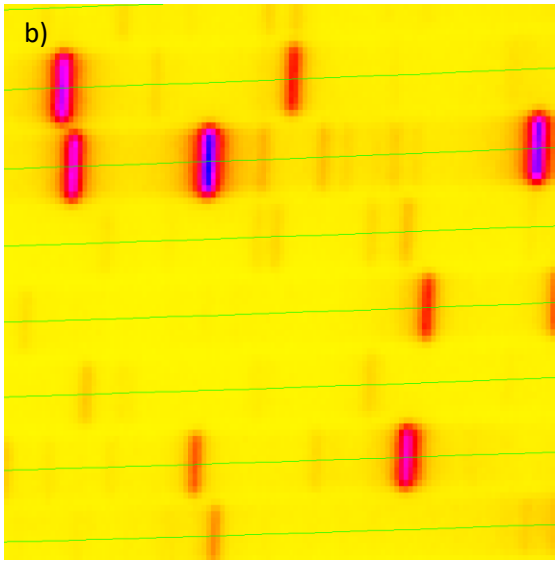
Total LIBS intensities
(sum of all emission line intensities)

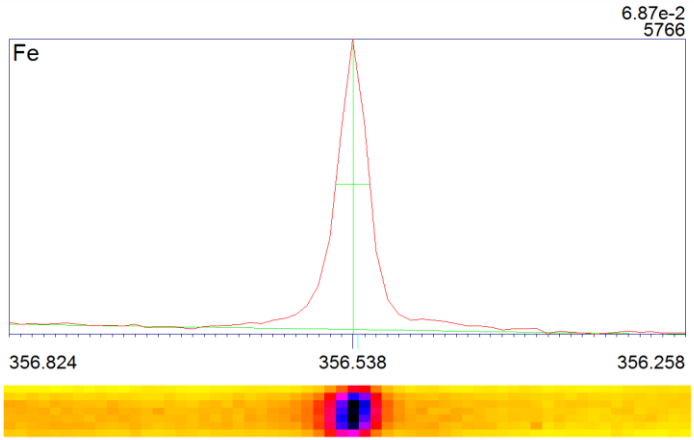
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
1																							
2		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18			
3																							
4	011518_initial_001	45031	5321	4725	484	1722	680	273	1275	51499	2462	29834	281	7592	1935	34879	16557	3597	3262	2632		214041	
5	011518_initial_002	24064	2880	1850	193	1033	368	121	391	19810	615	11223	109	5677	926	16852	9413	4563	4001	2896		106985	
6	011518_initial_003	48594	6016	4688	341	2234	833	391	1140	48821	2482	27579	340	7689	2012	35040	17209	3586	2405	2010		213410	
7	011518_initial_004	33761	3770	2485	272	1071	330	92	647	28840	1186	15149	150	5202	1102	19093	9314	3402	2205	1630		129701	
8	011518_initial_005	36198	5213	4127	372	1849	672	249	716	35778	1434	20598	218	7561	1554	28352	14636	4126	2872	2068		168593	
9	011518_initial_006	42666	4720	3682	458	1802	744	309	1310	51780	2618	28981	334	7819	2021	31160	14578	3859	2327	2090		203258	
10	011518_initial_007	32106	3459	2406	290	1103	542	212	385	19785	748	10420	121	4137	746	12335	6103	2115	1377	1062		99452	
11	011518_initial_008	34227	4212	2974	320	1316	503	231	642	31126	1223	17592	216	6830	1115	22205	11897	4391	3092	2445		146557	
12	011518_initial_009	32203	4069	2598	221	1299	499	218	618	29013	1370	15172	181	5427	1423	19143	10231	3284	1938	1571		130478	
13	011518_initial_010	40223	2756	1612	193	874	259	144	687	27948	1349	14586	129	2332	984	15682	7147	796	392	251		118344	
14	011518_initial_011	40941	4561	3159	333	1670	568	276	826	37600	1164	20946	162	7576	1552	29604	15680	5304	4164	3113		179199	
15	011518_initial_012	41961	5419	3616	471	1629	725	372	897	40183	1663	22672	188	6527	1469	29752	14917	3340	2287	1797		179885	
16	011518_initial_013	39016	2991	1757	176	774	303	265	644	24753	1350	12753	115	1929	369	10791	4972	567	351	201		104077	
17	011518_initial_014	46200	5778	3635	449	1823	623	414	844	43302	1615	24187	281	7738	1842	31749	15533	4176	3037	2417		195643	
18	011518_initial_015	42261	3947	2478	268	1120	432	265	1274	50880	2309	26391	220	5295	2009	29438	13274	1723	787	591		184962	
19	011518_initial_016	45051	5201	3500	353	1885	682	201	1198	49193	2209	27262	287	6655	2015	33046	15219	2691	1410	1182		199240	
20	011518_initial_017	35745	4263	3136	459	1416	618	239	791	30924	1286	17085	200	5687	1203	22172	11099	2793	2010	1369		142495	
21	011518_initial_018	42925	4331	3230	362	1470	596	301	1047	46231	2196	25803	188	6289	2375	28858	13276	2380	1340	1092		184290	
22	011518_initial_019	54679	3687	2779	355	1314	579	166	1205	51723	2309	27056	206	4836	2373	36628	16846	1730	1712	1289		211472	
23	011518_initial_020	37285	1880	1433	134	535	298	72	688	26305	1501	13475	197	2791	886	13484	6524	1559	2137	1574		112758	
24	Average	39756.9	4223.7	2993.5	325.2	1396.95	542.7	240.55	861.25	37274.7	1654.45	20438.2	206.15	5779.45	1495.55	25013.2	12221.3	2999.1	2155.3	1664			
25	STDEV	6856.36	1093.51	947.017	106.339	432.732	165.313	93.0605	292.378	11243.4	595.94	6441.19	68.7852	1834.73	567.506	8286.22	3891.36	1288.32	1058.16	816.58			
26	Normalized Wavelengths																						
27	011518_initial_001	0.21038	0.02486	0.02208	0.00226	0.00805	0.00318	0.00128	0.00596	0.2406	0.0115	0.13938	0.00131	0.03547	0.00904	0.16295	0.07735	0.01681	0.01524	0.0123			
28	011518_initial_002	0.22493	0.02692	0.01729	0.0018	0.00966	0.00344	0.00113	0.00365	0.18517	0.00575	0.1049	0.00102	0.05306	0.00866	0.15752	0.08798	0.04265	0.0374	0.02707			
29	011518_initial_003	0.2277	0.02819	0.02197	0.0016	0.01047	0.0039	0.00183	0.00534	0.22877	0.01163	0.12923	0.00159	0.03603	0.00943	0.16419	0.08064	0.0168	0.01127	0.00942			
30	011518_initial_004	0.2603	0.02907	0.01916	0.0021	0.00826	0.00254	0.00071	0.00499	0.22236	0.00914	0.1168	0.00116	0.04011	0.0085	0.14721	0.07181	0.02623	0.017	0.01257			
31	011518_initial_005	0.21471	0.03092	0.02448	0.00221	0.01097	0.00399	0.00148	0.00425	0.21222	0.00851	0.12218	0.00129	0.04485	0.00922	0.16817	0.08681	0.02447	0.01704	0.01227			
32	011518_initial_006	0.20991	0.02322	0.01811	0.00225	0.00887	0.00366	0.00152	0.00645	0.25475	0.01288	0.14258	0.00164	0.03847	0.00994	0.1533	0.07172	0.01899	0.01145	0.01028			
33	011518_initial_007	0.32283	0.03478	0.02419	0.00292	0.01109	0.00545	0.00213	0.00387	0.19894	0.00752	0.10477	0.00122	0.0416	0.0075	0.12403	0.06137	0.02127	0.01385	0.01068			
34	011518_initial_008	0.23354	0.02874	0.02029	0.00218	0.00898	0.00343	0.00158	0.00438	0.21238	0.00834	0.12004	0.00147	0.0466	0.00761	0.15151	0.08118	0.02996	0.0211	0.01668			

Total LIBS intensity
(for 20th file)

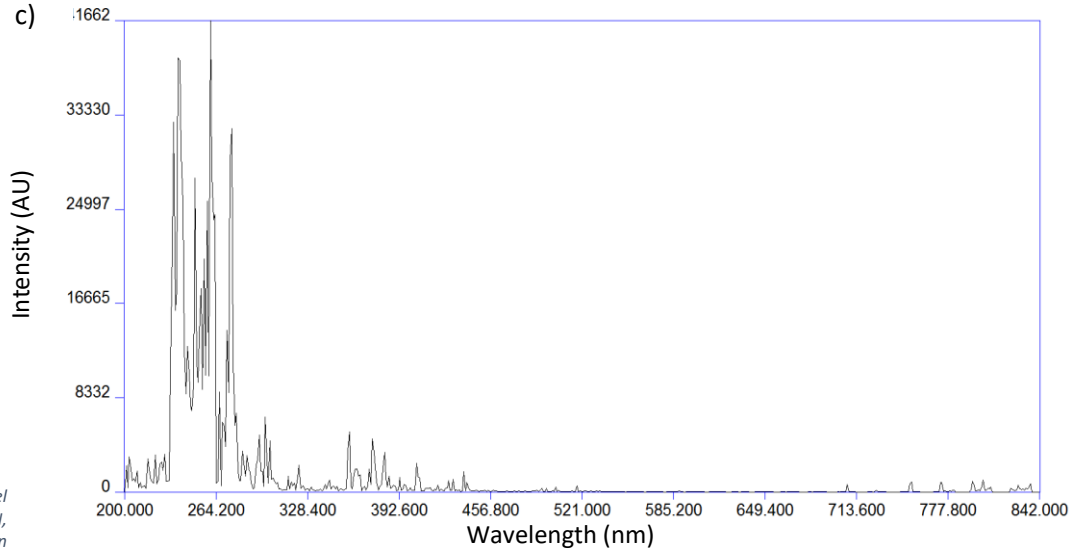




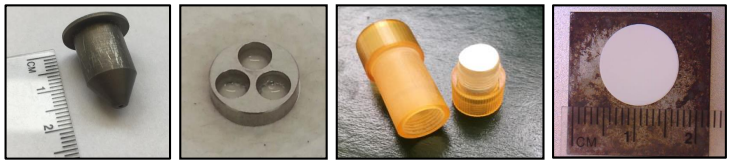




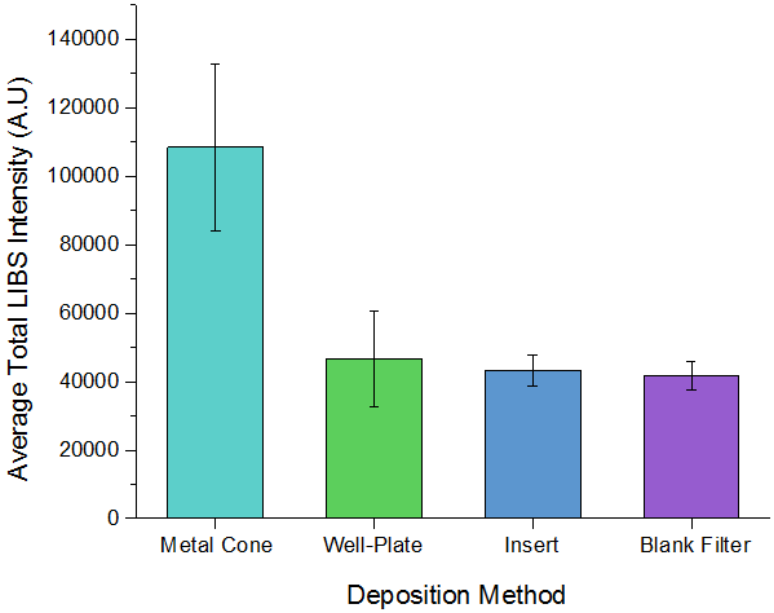
ROI view from ESAWIN software. The line plot in red is the intensity as a function of the X-pixel coordinates for 60 pixels. The vertical green line depicts the center of the peak according to ESAWIN, and the blue line below and to the right of the vertical green line shows the expected location of an emission line according to the NIST atomic database. The horizontal green lines designate the background and the FWHM. The text in the upper left corner denotes the element. The numbers in the upper right, from top to bottom, denote the ratio of the peak area to some reference line (not used in this work) and the peak area. Numbers below the window are wavelengths in nm. Below the window shows the portion of the échellogram corresponding to that ROI.



Comparison of LIBS Signal to Previous Deposition Methods

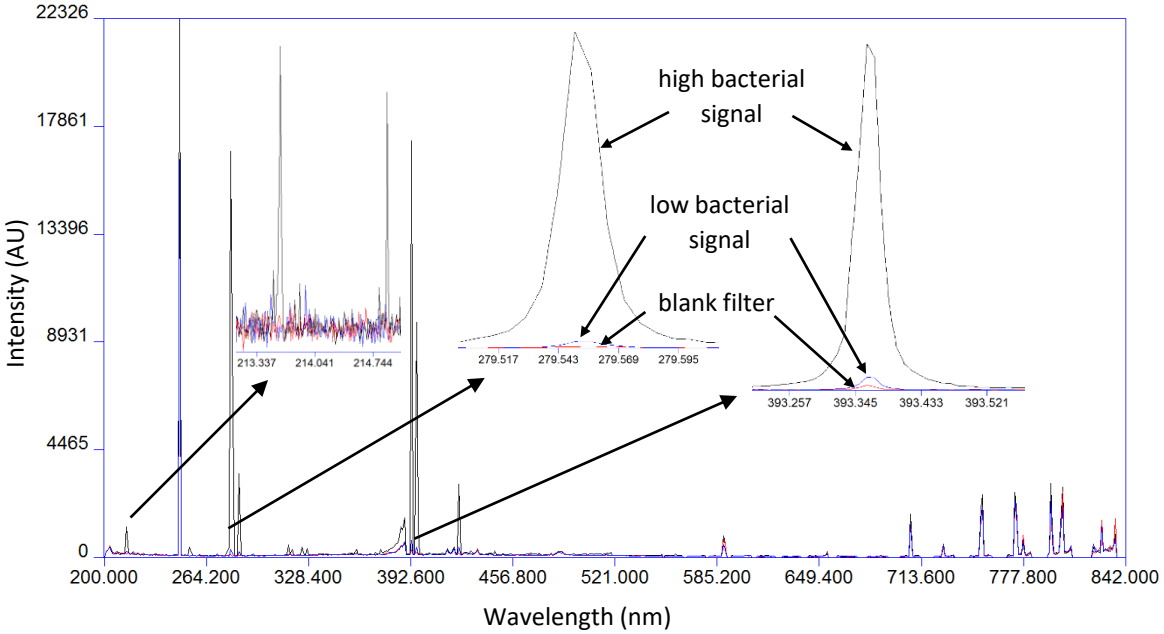


metal cone | well-plate | insert | blank filter



Suggestive of a **lower LOD** for bacteria deposited with metal cone

Efforts to Reduce Shot-to-Shot Variations in LIBS Signal of *E. coli* Cells

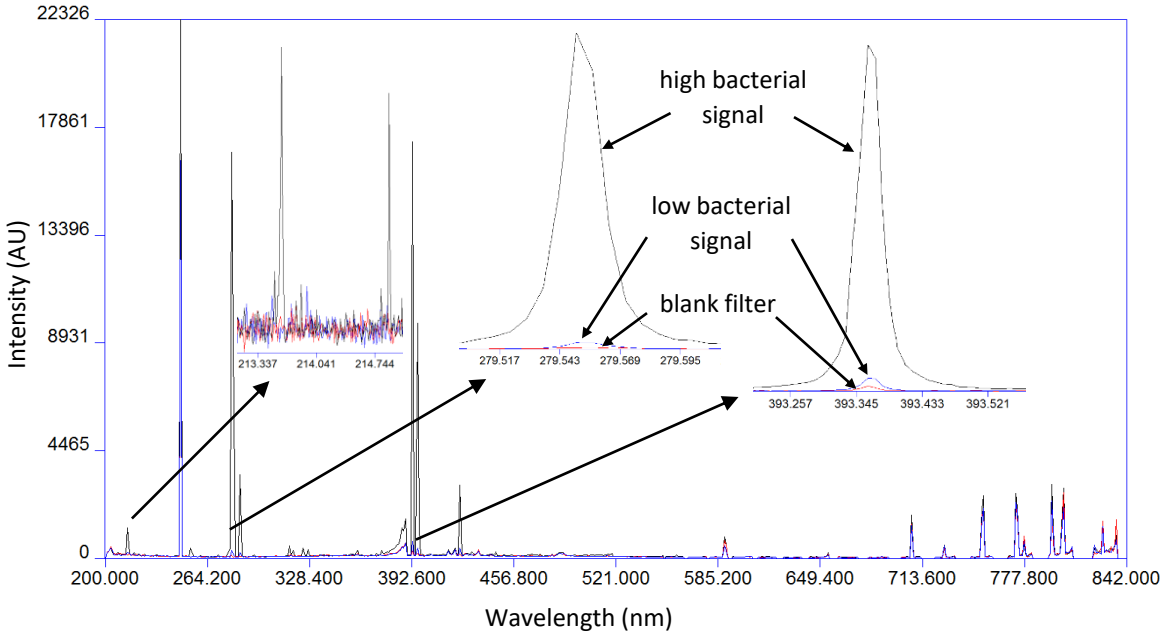


Two overlapped E. coli spectra taken side-by-side on the same filter paper, showing evidence of non-uniform laser ablation. Black spectrum exhibits high bacterial signal and blue spectrum exhibits signal comparable to a blank filter which is shown in red. Insets show zoomed-in sections of the emissions from phosphorus, magnesium, and calcium

- Effect of **Tween 20** in LIBS analysis of *E. coli*
- Effect of growth of *E. coli* in **liquid medium** on LIBS analysis

NOT EFFECTIVE

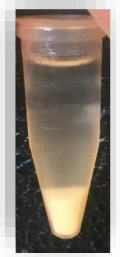
Effect of Tween 20 in LIBS Analysis of *E. coli*



Two overlapped E. coli spectra taken side-by-side on the same filter paper, showing evidence of non-uniform laser ablation. Black spectrum exhibits high bacterial signal and blue spectrum exhibits signal comparable to a blank filter which is shown in red. Insets show zoomed-in sections of the emissions from phosphorus, magnesium, and calcium

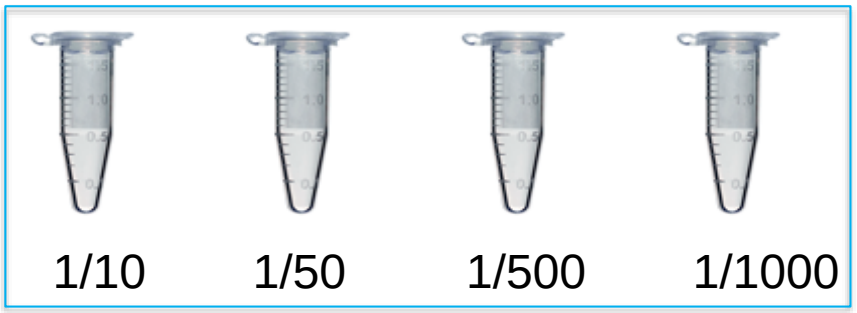
- Tween 20 is a detergent
- Detergents make compounds that are insoluble in water miscible in aqueous media
- Treatment with Tween 20 may aid in distributing cells more evenly throughout, preventing bacteria from forming clumps

Provide a more consistent LIBS bacterial signal?

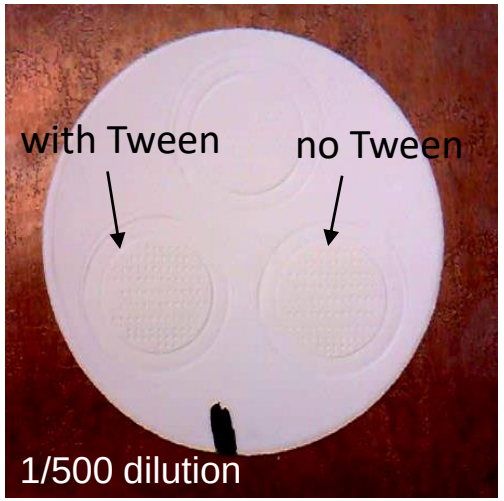


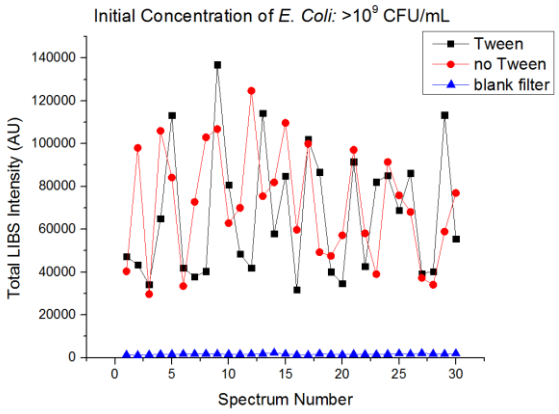
Initial suspension of *E. coli*

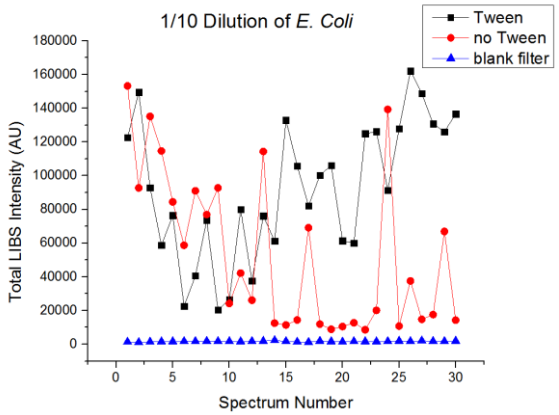
test **with** Tween
(0.1% concentration of Tween)

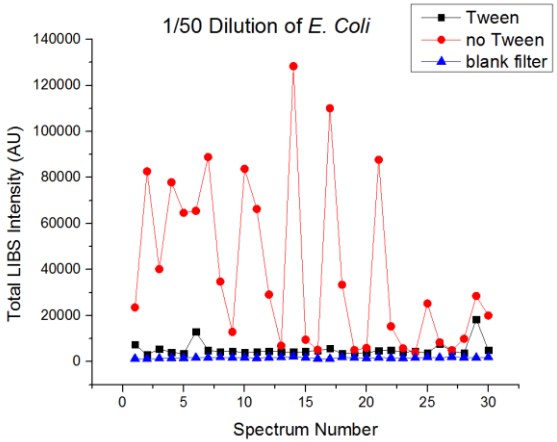


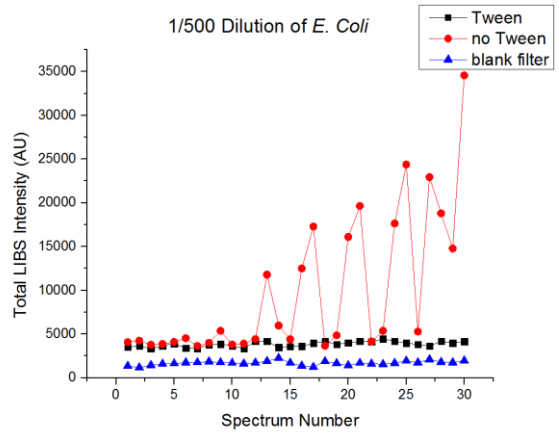
test **without** Tween

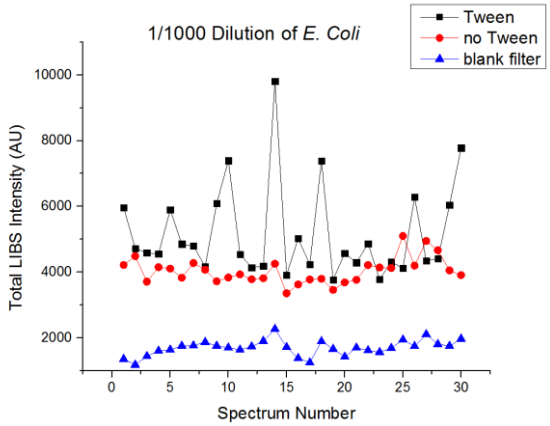


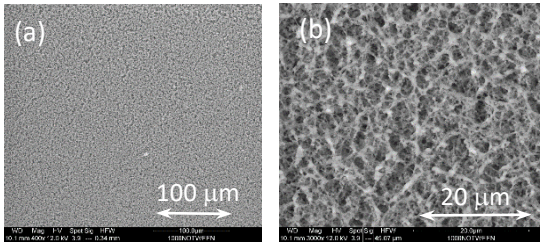

Initial Concentration of E. Coli: >10^9 CFU/mL
Tween
no Tween
blank filter
Total LIBS Intensity (AU)
Spectrum Number


1/10 Dilution of E. Coli
Tween
no Tween
blank filter
Total LIBS Intensity (AU)
Spectrum Number

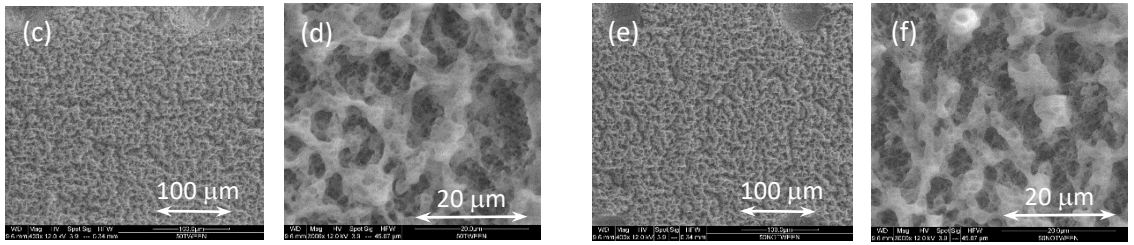

1/50 Dilution of E. Coli
Tween
no Tween
blank filter
Total LIBS Intensity (AU)
Spectrum Number


1/500 Dilution of E. Coli
Tween
no Tween
blank filter
Total LIBS Intensity (AU)
Spectrum Number


1/1000 Dilution of E. Coli
Tween
no Tween
blank filter
Total LIBS Intensity (AU)
Spectrum Number

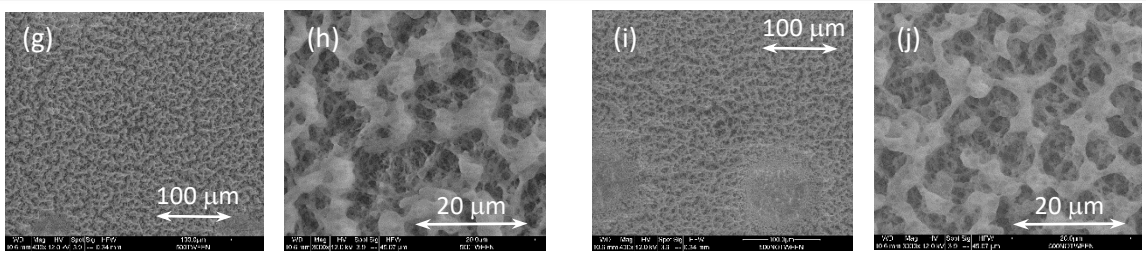


Filter paper



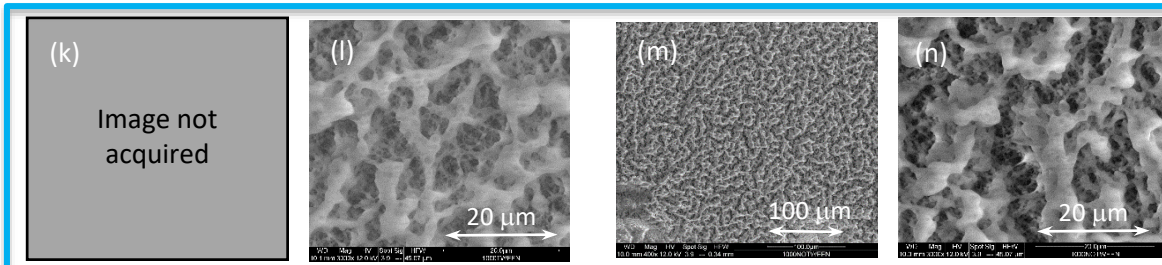
1/50 (tween)

1/50 (no tween)



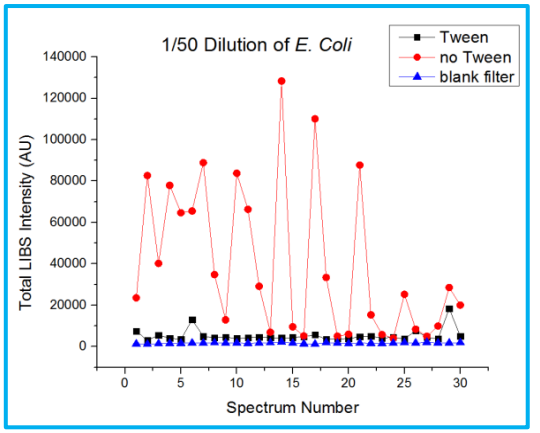
1/500 (tween)

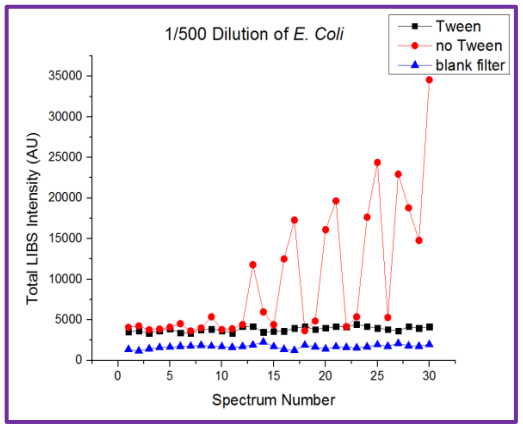
1/500 (no tween)

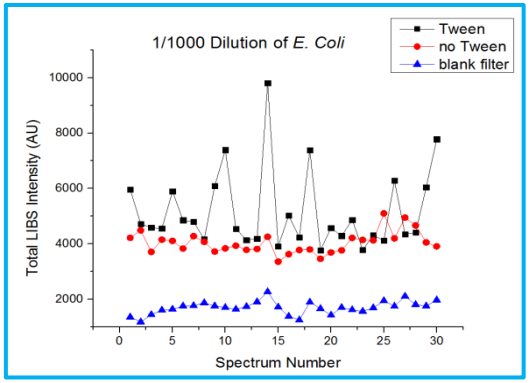


1/1000 (tween)

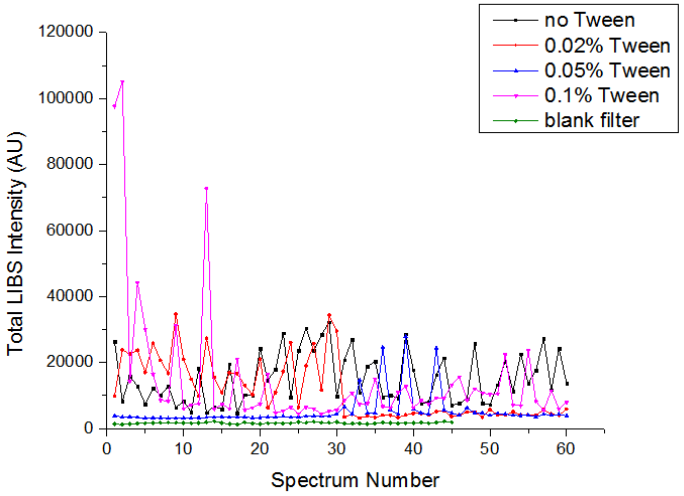
1/1000 (no tween)

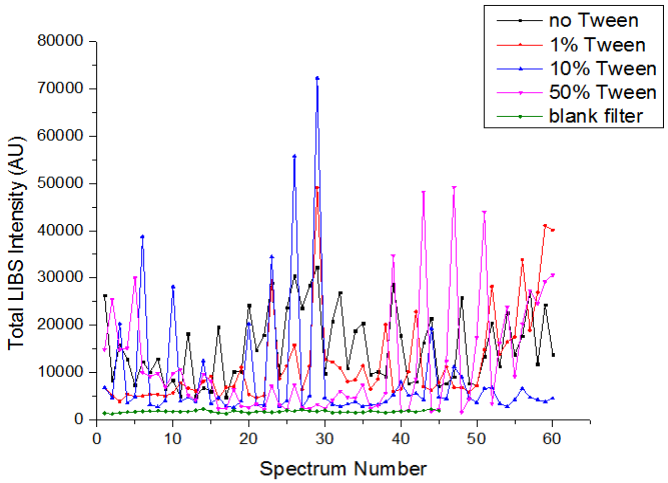


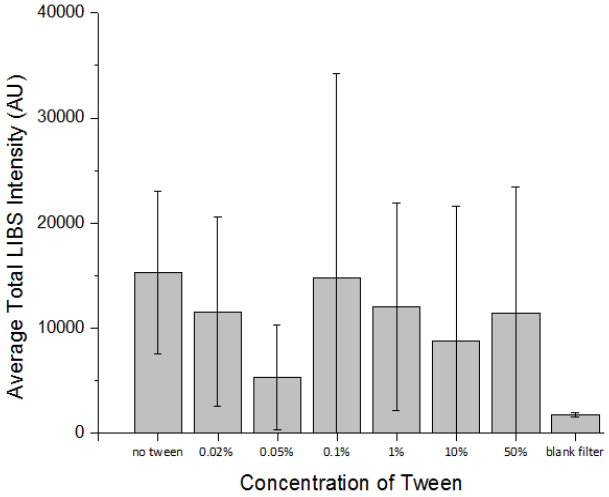




Is Tween only effective at a certain concentration?



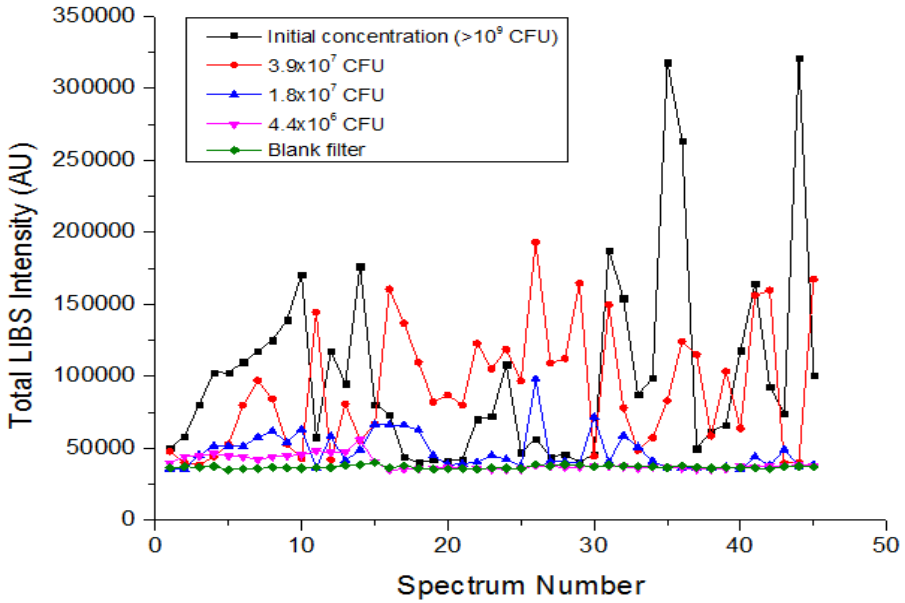




- None of the concentrations of Tween used improved the shot-to-shot variations in bacterial LIBS signal
- Tween may only be effective on certain species of bacteria

Effect of Growing *E. coli* in Liquid Medium on LIBS Analysis

- Bacteria grow dispersed in liquid media
- More closely resembles growth of bacteria in the body
- Provide a more consistent LIBS bacterial signal?



- Growth of *E. coli* in liquid medium was ineffective at improving shot-to-shot variations
- Improve the incubation procedure