Laser-Induced Breakdown Spectroscopy as a Rapid Diagnostic Tool for Bacterial Detection and Discrimination

Alexandra Paulick, Steven Rehse, Dylan Malenfant,
Kevin Beaugrand, Mark Armstrong

Motivation

- Current methods of bacterial identification in a clinical setting
  - require expertise in microbiology
  - labor-intensive
  - 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**

GOAL:

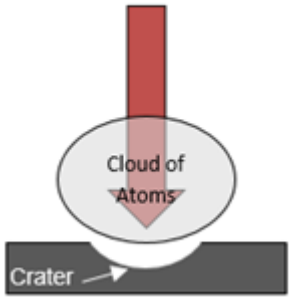
Rapidly identify bacteria based on their elemental composition using laser-induced breakdown spectroscopy (LIBS)

This includes developing a **quick bacterial preparation method** prior to testing that utilizes equipment and methods that are **common in a clinical setting**

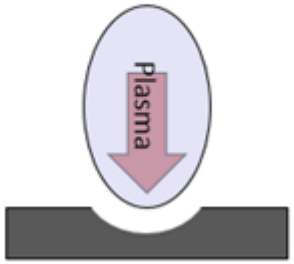
Laser-Induced Breakdown Spectroscopy (LIBS)

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

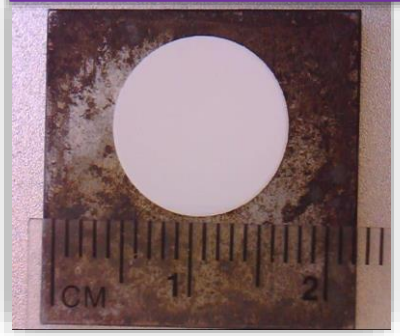
- Can be done on **solids**, **liquids**, **gases** and **bacteria**
- Little to no sample preparation
- Requires only **μg** of sample
- **Fast:** elemental composition can be determined in under *1 second*
- Simultaneously detects **all elements in periodic table**
- The use of the laser allows for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

Overview of Methodology

1 Bacteria is cultured on TSA plates

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

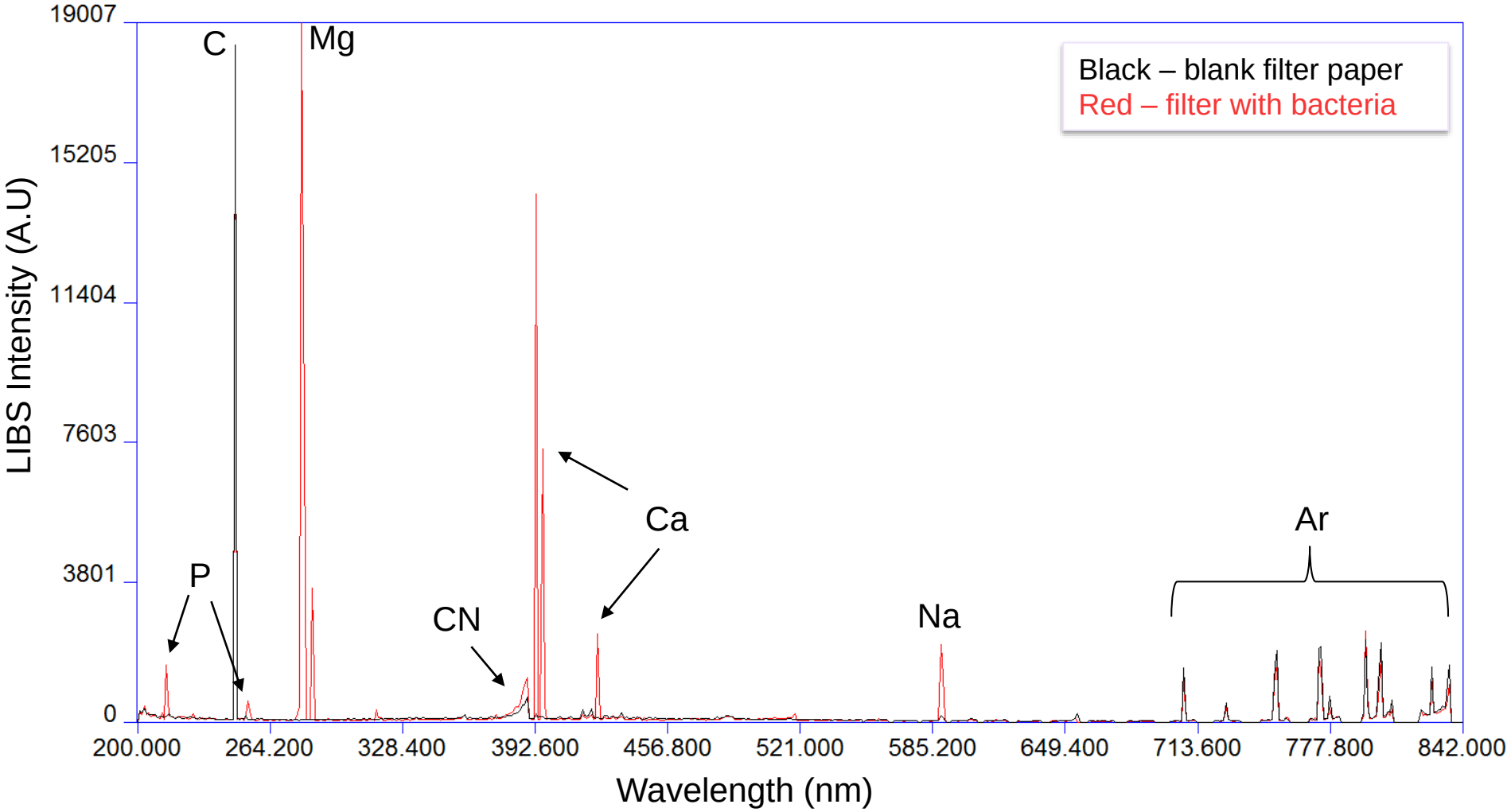
3 Bacterial suspension is vortexed and deposited on nitrocellulose filter paper



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



6 Bacteria are discriminated based on elemental composition

C, P, Mg, Ca, and Na can be used to identify a bacterium's species

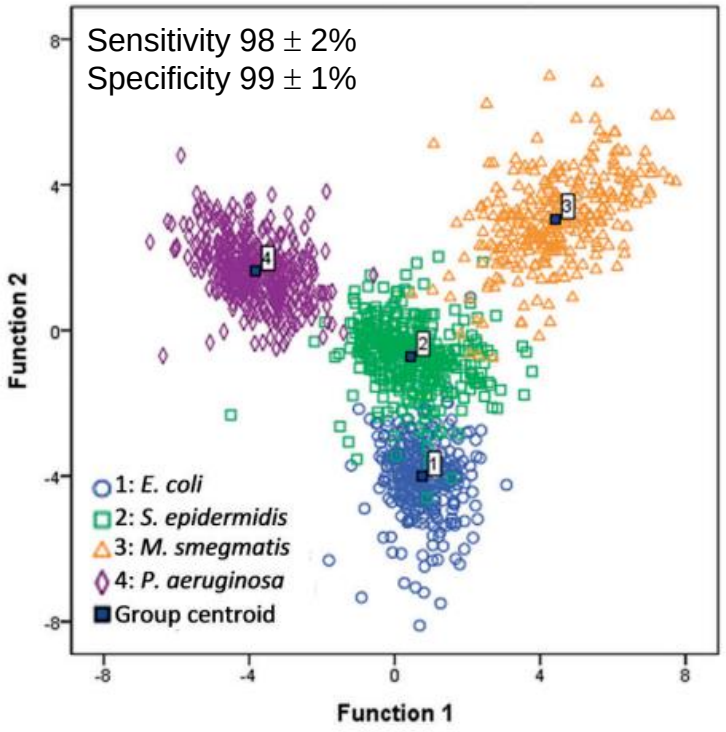
Bacterial spectra are classified using discriminant function analysis (**DFA**)

- 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

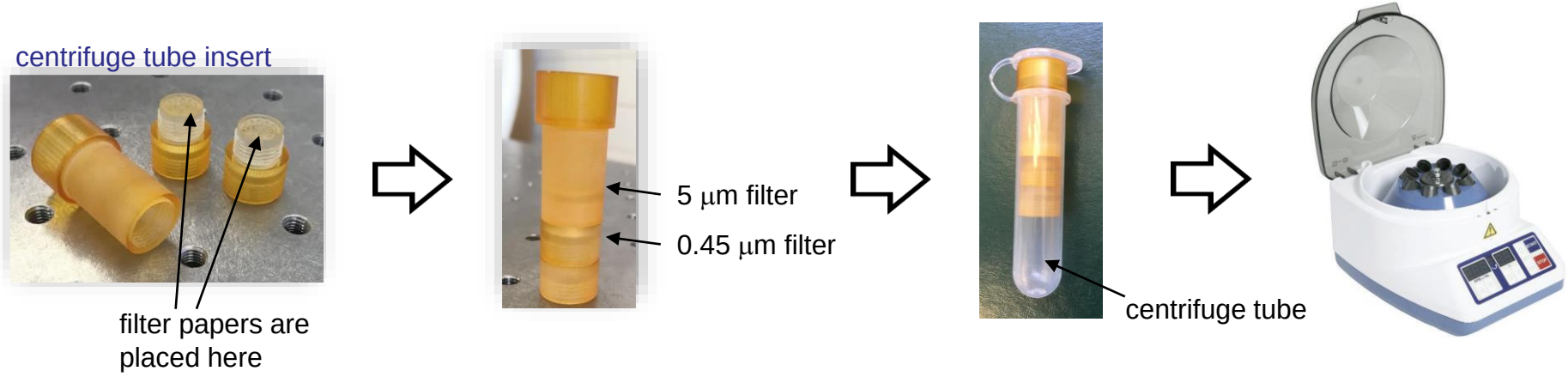


Preparation Method to Separate a Contaminant from a Bacterial Suspension

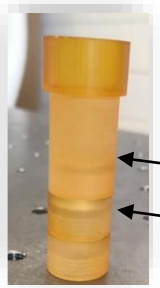
Biological samples (blood sample, swab sample, etc.) will likely contain **unwanted cells** that would need to be separated from the bacteria before testing with LIBS

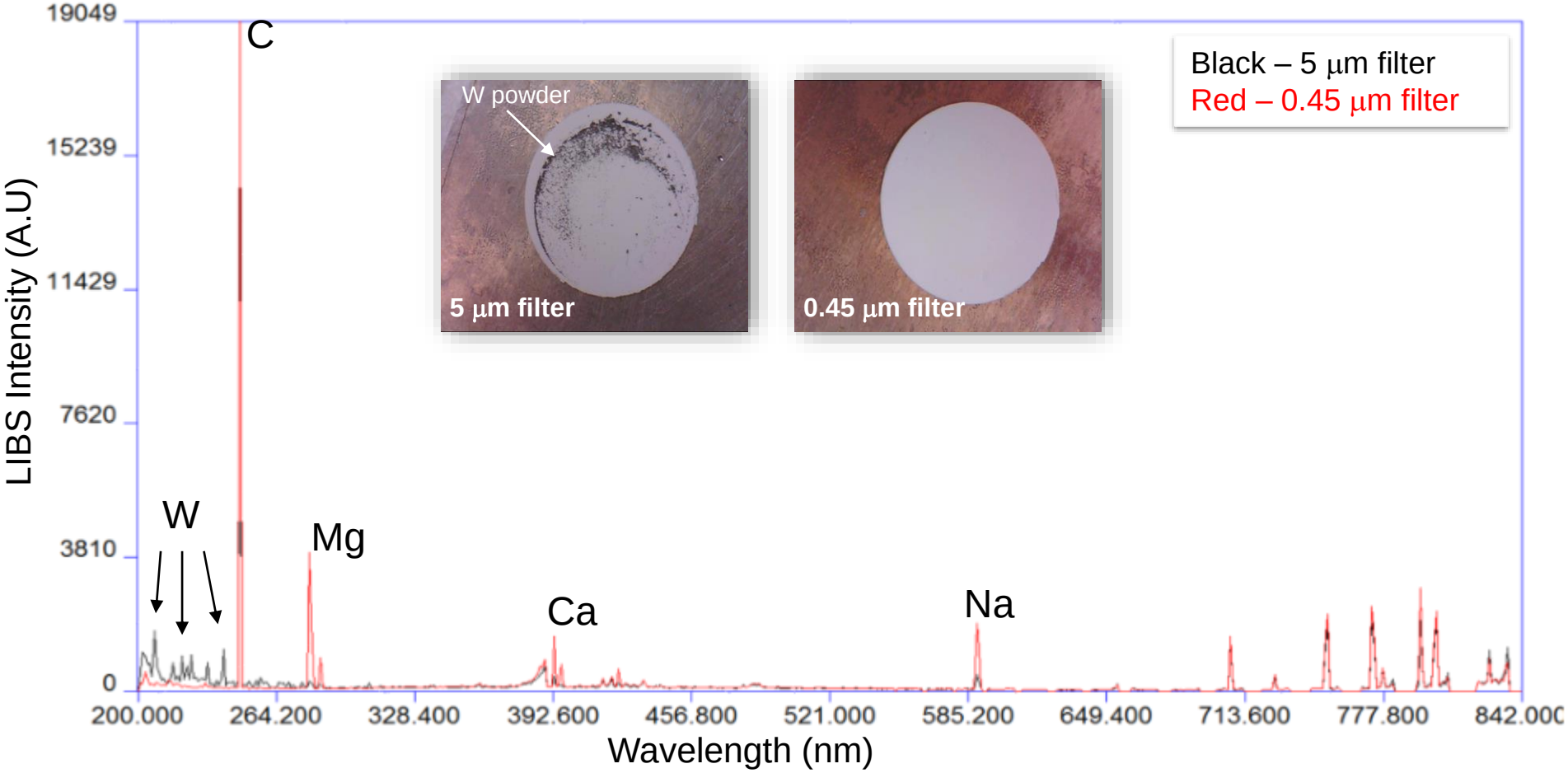
Cell sizes:
- Bacteria ~ 1 μm
- Red blood cell ~ 6-8 μm
- Eukaryotic cells ~ 10-100 μm

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



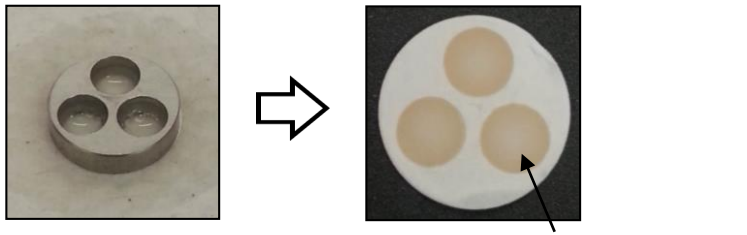
Tungsten powder (12 μm average particle size) added to *E. coli* suspension to simulate unwanted cells in a biological sample





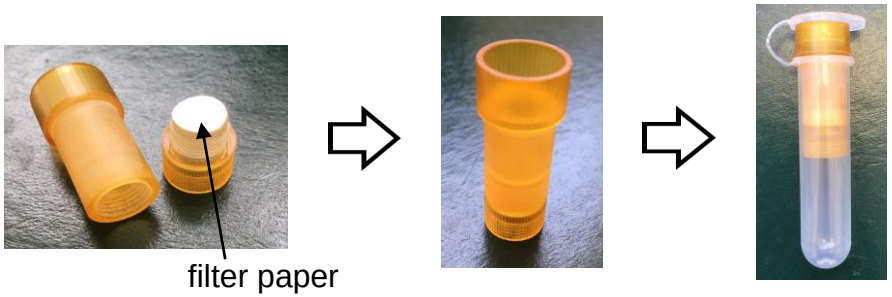
Previous Bacterial Deposition Procedures

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

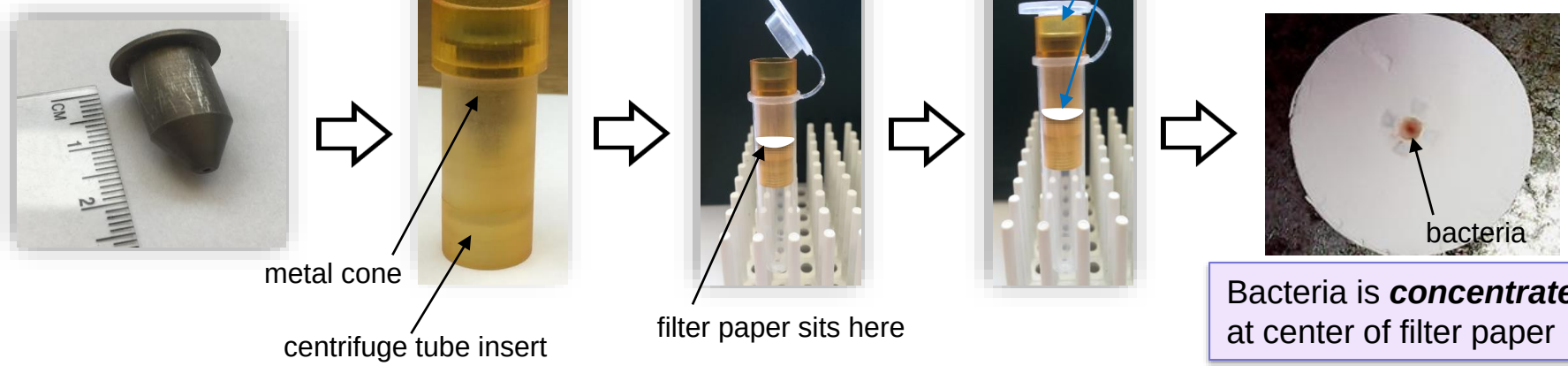
These LOD's are ***not clinically relevant***. Bacterial LOD with LIBS MUST be lowered.

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

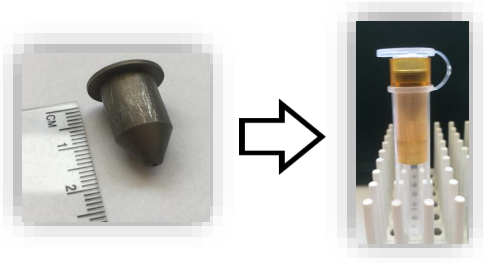
Method:



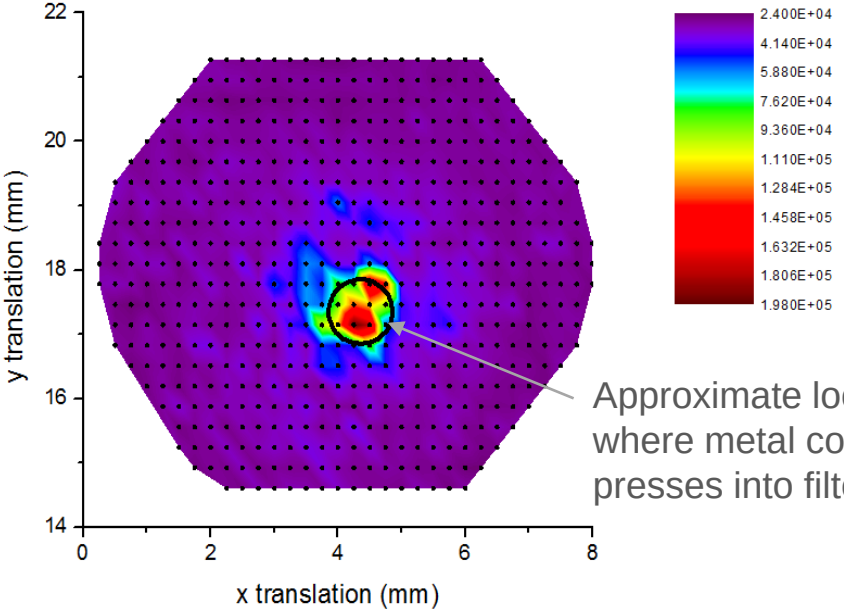
Bacteria is ***concentrated*** at center of filter paper

Metal Cone: Bacterial Concentration

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



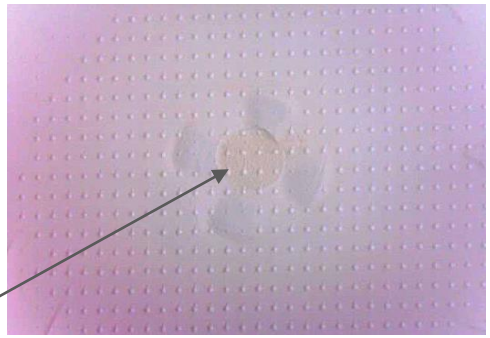
Intensity map depicting bacterial deposition on filter paper for bacteria deposited with metal cone



Approximate location of where metal cone presses into filter paper

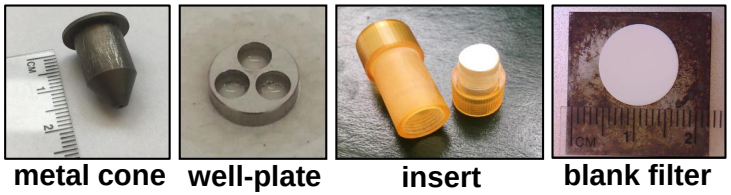
Colour indicates bacterial LIBS intensity
→ purple: no bacterial signal
→ red: strong bacterial signal

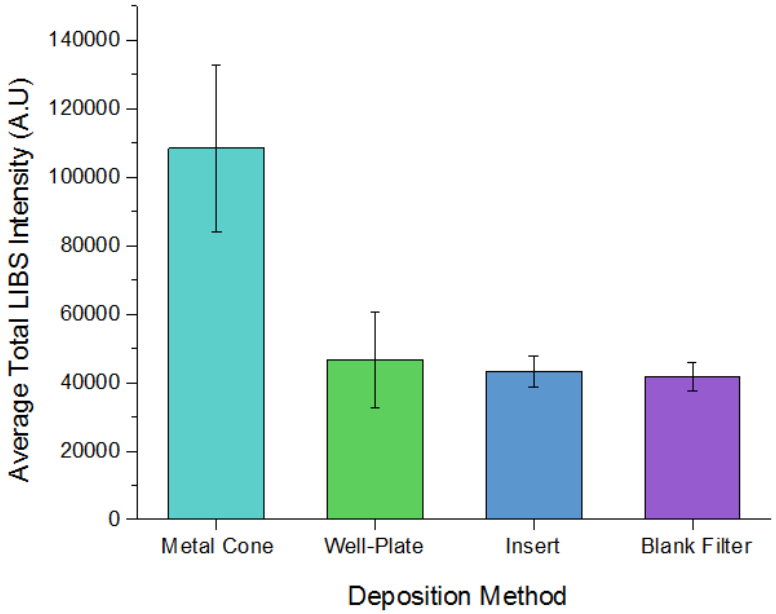
Image of filter paper after data acquisition



Discolouration due to presence of bacteria

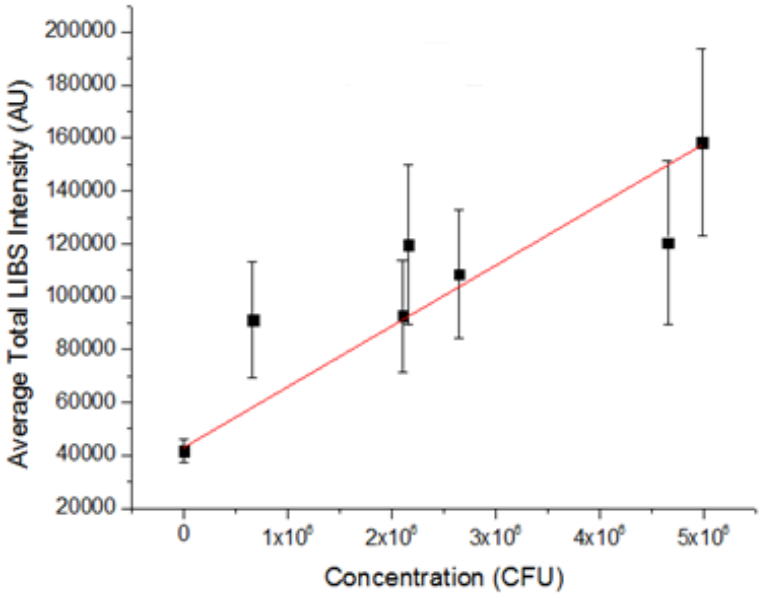
Comparison of LIBS Signal to Previous Deposition Methods





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

Metal Cone Limit of Detection



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

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

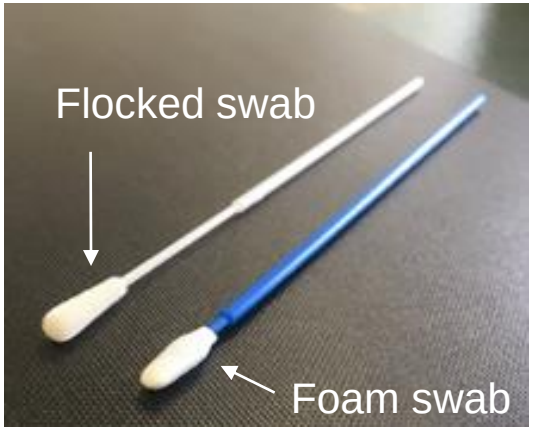
Conclusions

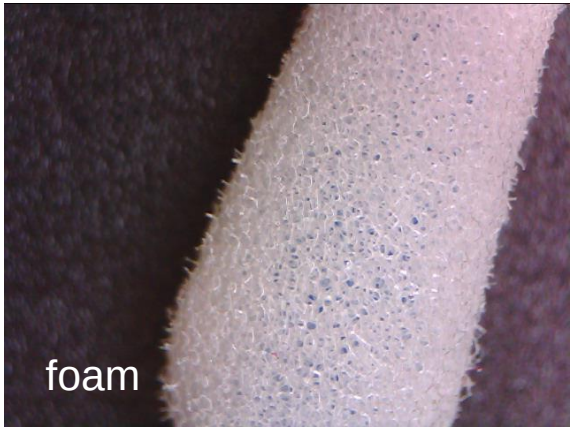
- Preparation method to separate unwanted material from bacterial suspension was effective

  Future work: test this method using a contaminant that more closely simulates biological cells

- Metal cone:
  - effective at concentrating bacterial cells to a small region of the filter paper
  - significantly lowered bacterial LOD with LIBS compared to previous methods of bacterial deposition

- **Future work:** LIBS analysis on bacteria collected with swabs that are used in hospitals

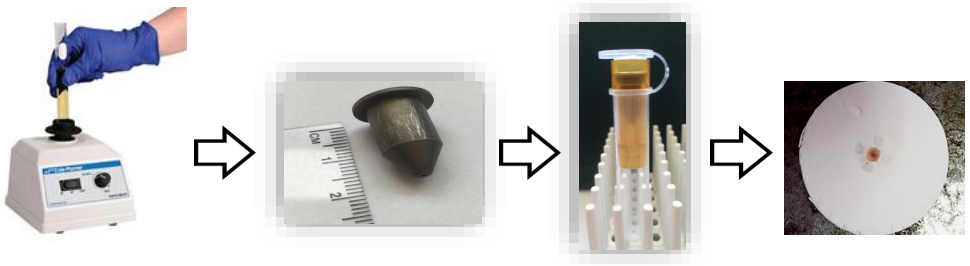


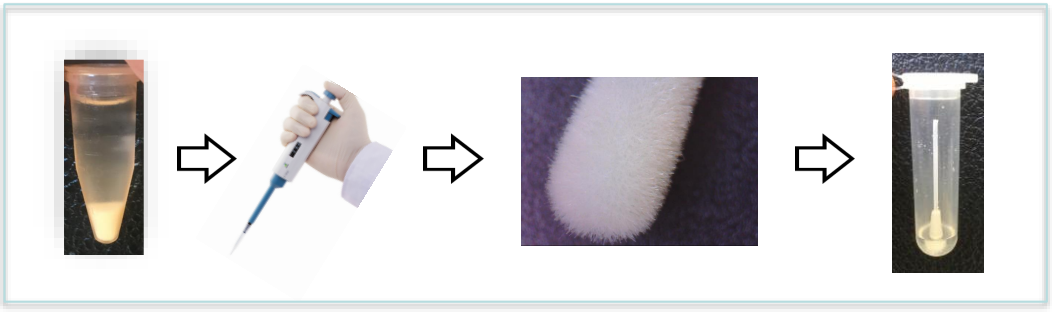


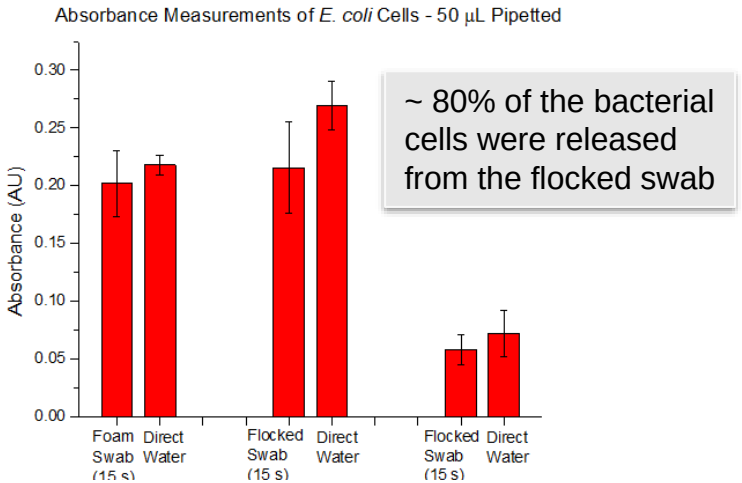
Cannot shoot right on the swab

→ Surface is too irregular

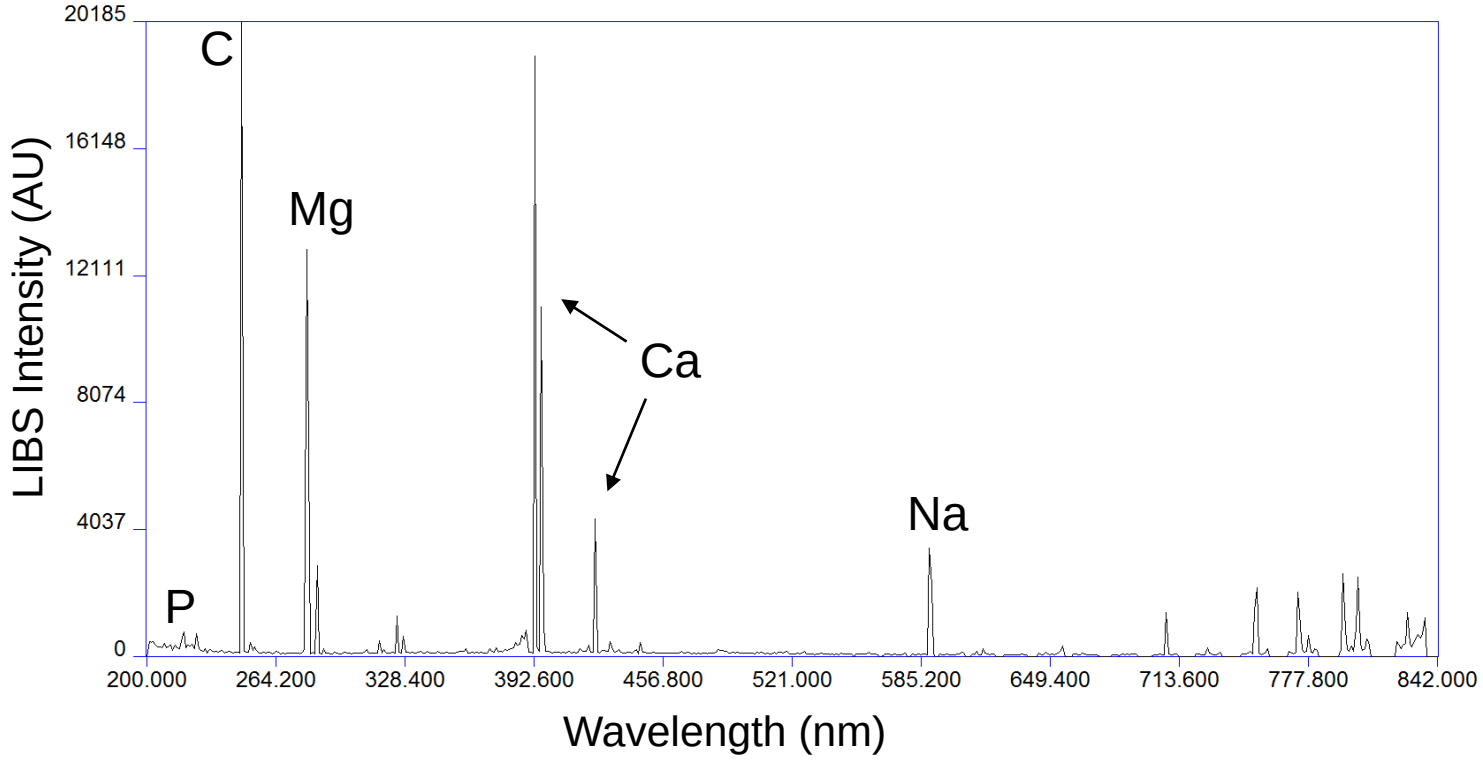
→ Bacterial cells are not concentrated







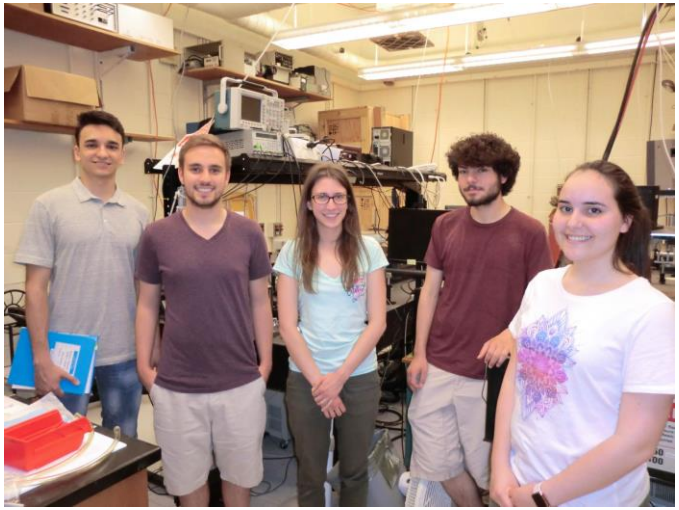
~ 80% of the bacterial cells were released from the flocked swab



Acknowledgments

- NSERC 

- University of Windsor Student Life Enhancement Fund
- University of Windsor Faculty of Science

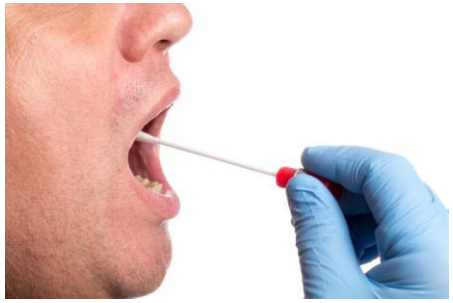


New students welcome!

If interested, contact Dr. Steven Rehse
rehse@uwindsor.ca

Bacterial Collection with Swabs

Some clinical specimens are collected with 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?