Healing Humanity One Spark at a Time: Diagnosing Bacterial Pathogens with Laser-Induced Breakdown Spectroscopy

Presented at Michigan Technological University
Thursday, April 6th, 2023

Steven J. Rehse

Department of Physics
University of Windsor

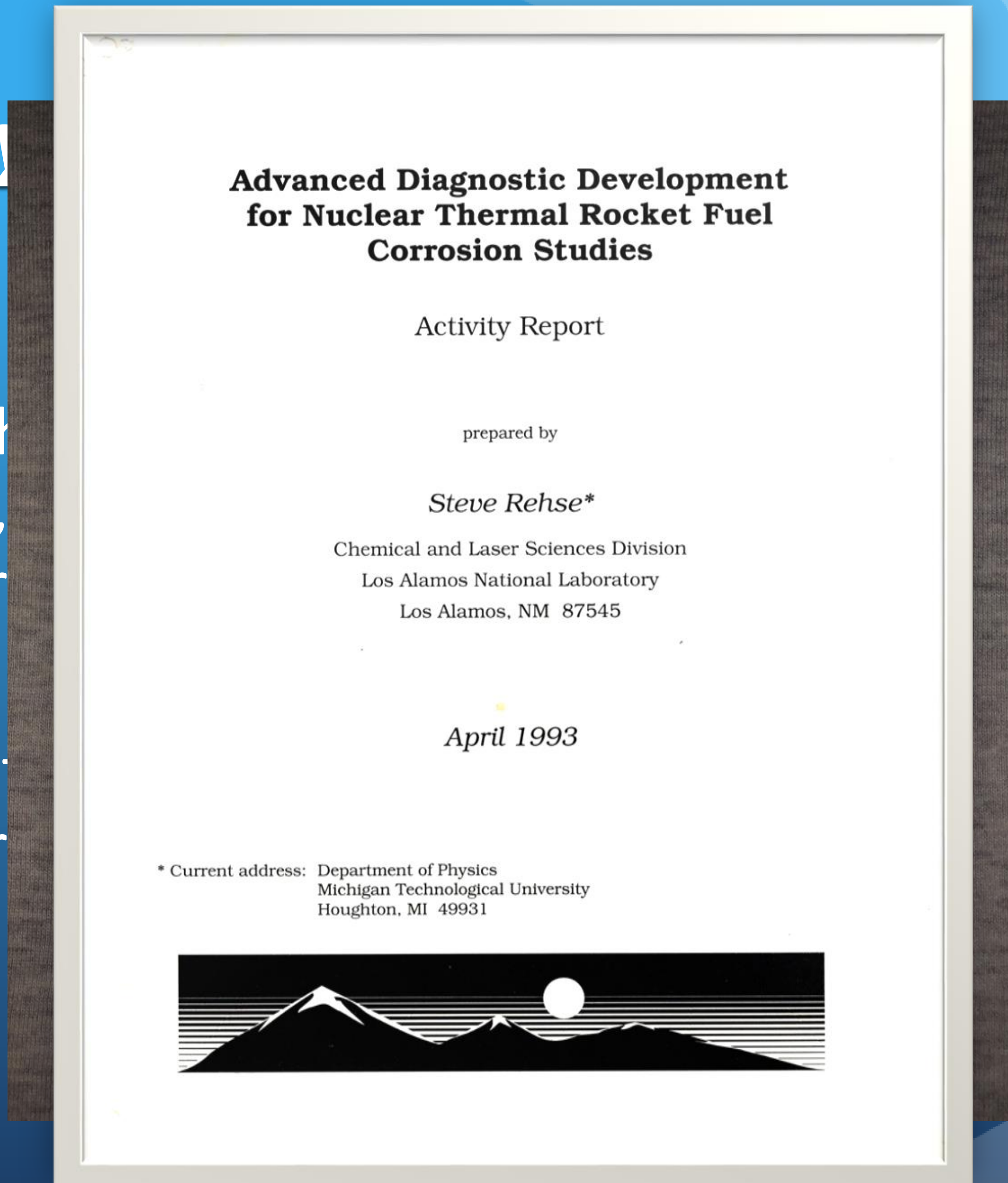
History

1. Started

2. Took a h… om August, quarter

3. Gradua… up two quarter

4. Moved



Advanced Diagnostic Development for Nuclear Thermal Rocket Fuel Corrosion Studies

Activity Report

prepared by

*Steve Rehse**

Chemical and Laser Sciences Division
Los Alamos National Laboratory
Los Alamos, NM 87545

April 1993

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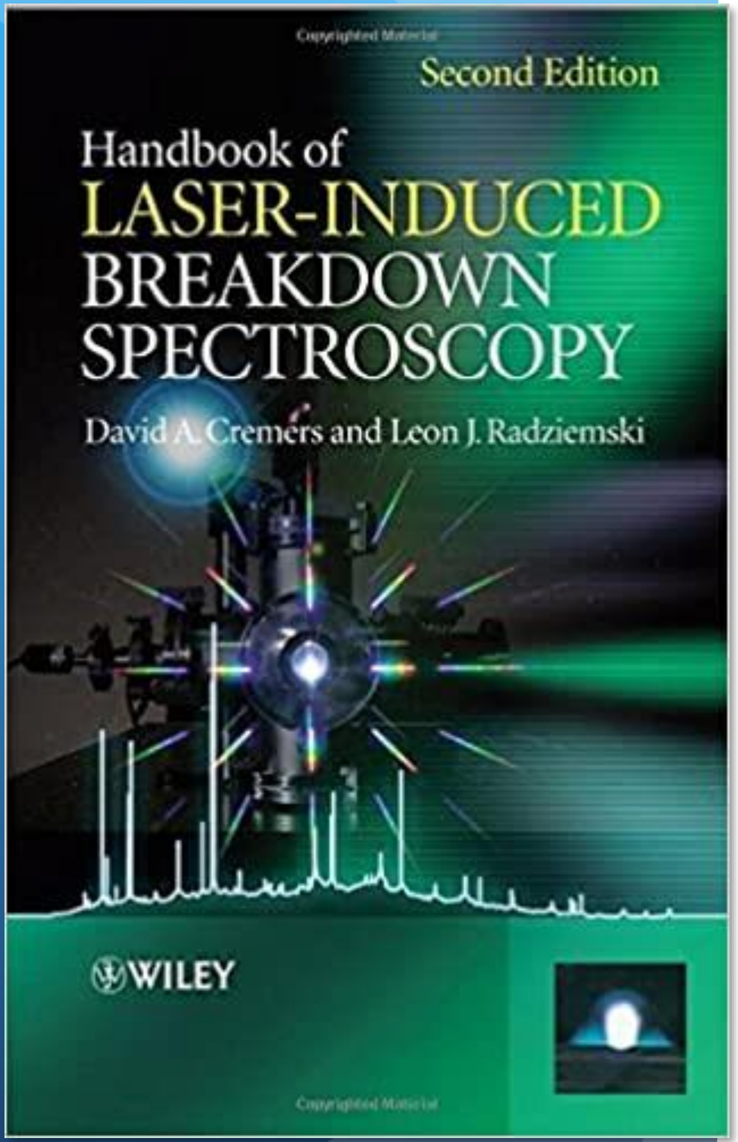
Michigan Technological University
Houghton, MI 49931

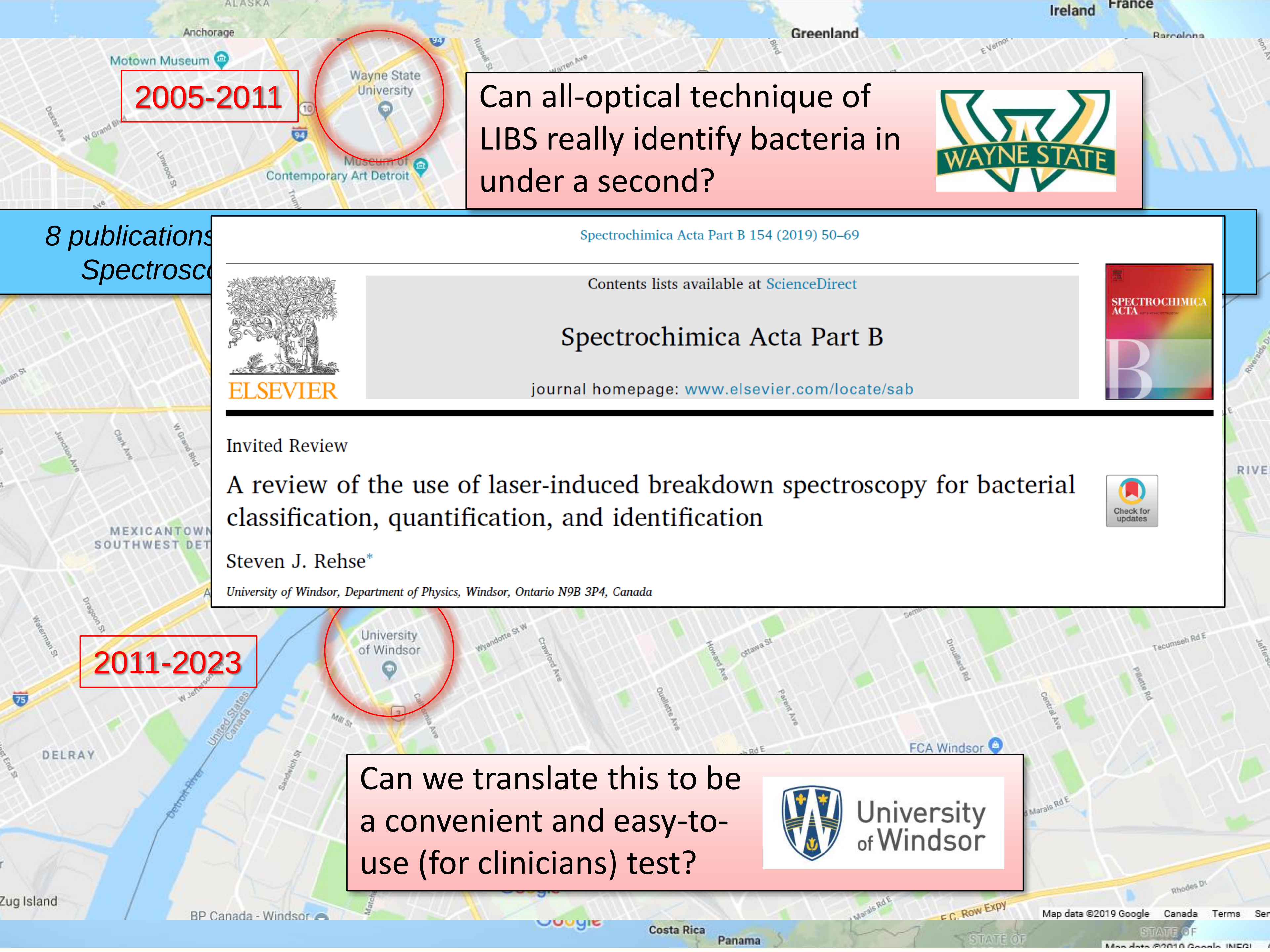
A Neat Coincidence

1. I worked in the "Chemical and Laser Science (CLS) Division" - later renamed the "Chemical Science and Technology (CST) Division."

2. I worked across the hall from Dr. David Cremers, who was busy bringing "LIBS" to prominence.

3. 10 years later I would begin using the technique myself.





2005-2011

Can all-optical technique of LIBS really identify bacteria in under a second?

WAYNE STATE

8 publications
Spectrosc

Spectrochimica Acta Part B 154 (2019) 50–69

Contents lists available at ScienceDirect

Spectrochimica Acta Part B

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

ELSEVIER

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

2011-2023

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

University of Windsor

Outline

1. Introduction of the Method.
   Laser-induced breakdown spectroscopy (LIBS)

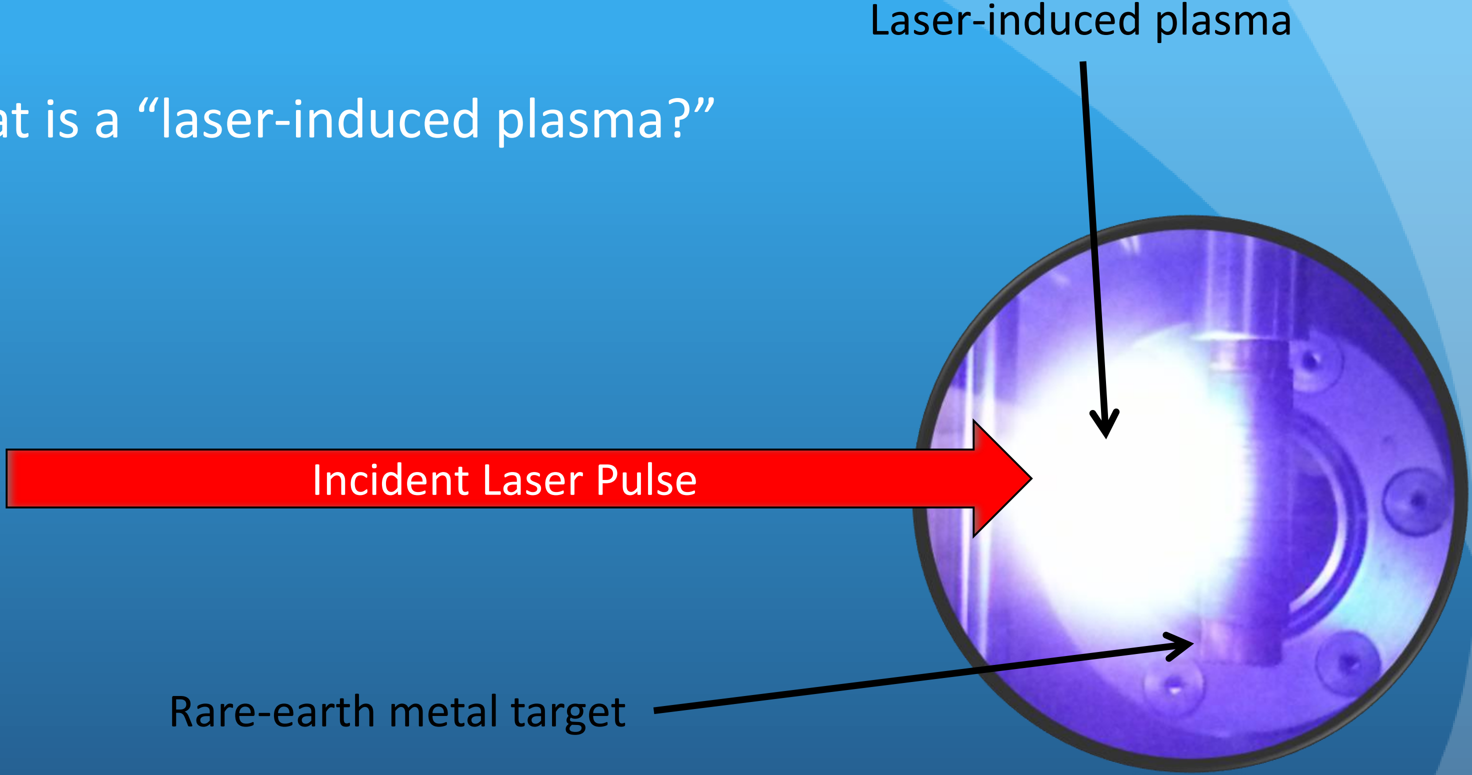
2. Advantages of LIBS

3. Biomedical Applications of LIBS: A new paradigm for rapid pathogen identification

4. Concluding Thoughts

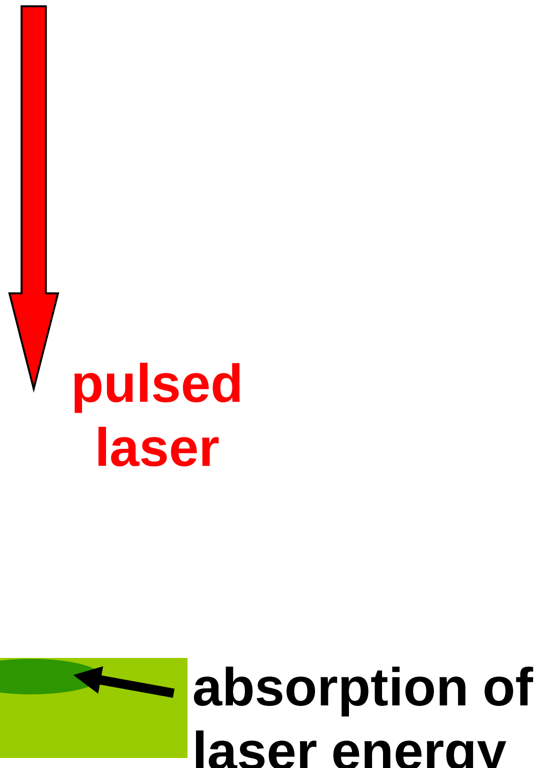
Laser-Induced Plasmas

- What is a "laser-induced plasma?"



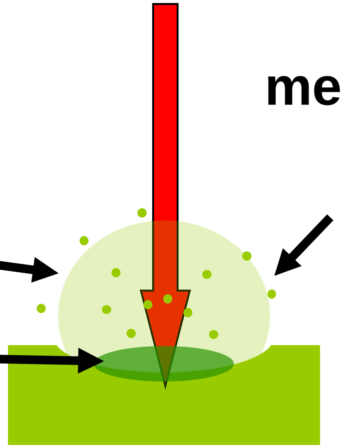
- Can be done with ns, ps, or fs lasers
- Threshold irradiance: $10^{10} - 10^{11}\ \mathrm{W/cm^2}$

1) laser interaction with the target



- initiated by absorption of energy by the target from a pulsed radiation field.
- pulse durations are on the order of nanoseconds, but can be performed with pico- and femto-second laser pulses.

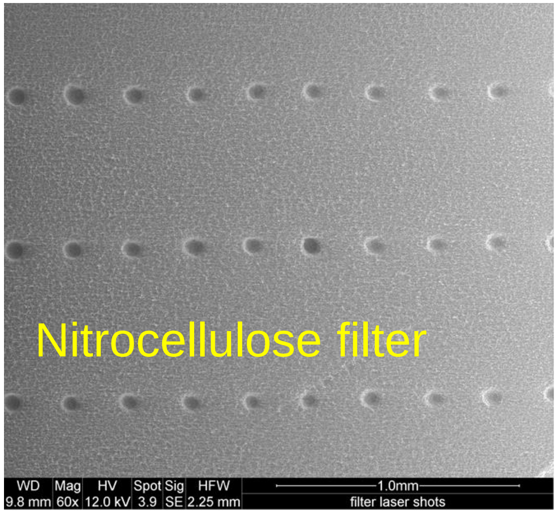
2) removal of samples mass (ablation)

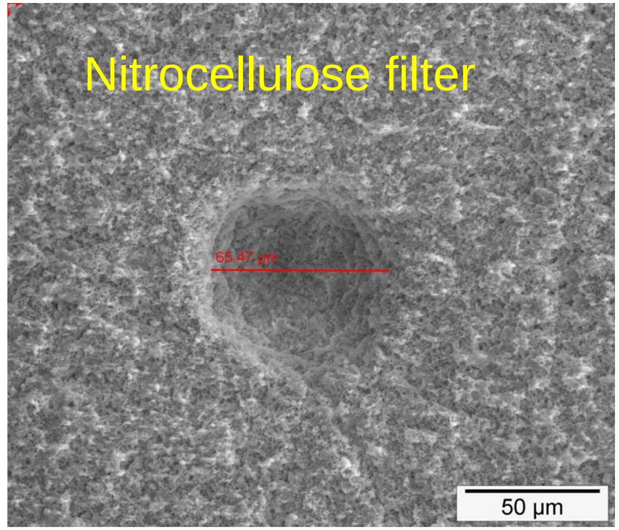


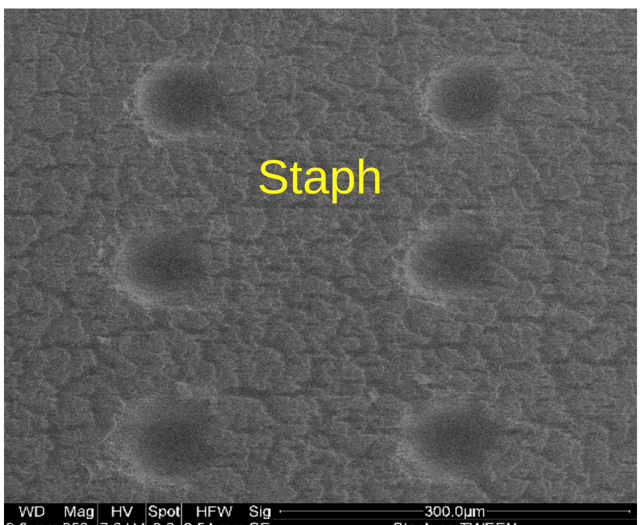
- absorbed energy is rapidly converted into heating, resulting in vaporization of the sample (*ablation*) when the temperature reaches the boiling point of the material.

- removal of particulate matter from the surface leads to the formation of a vapor above the surface.

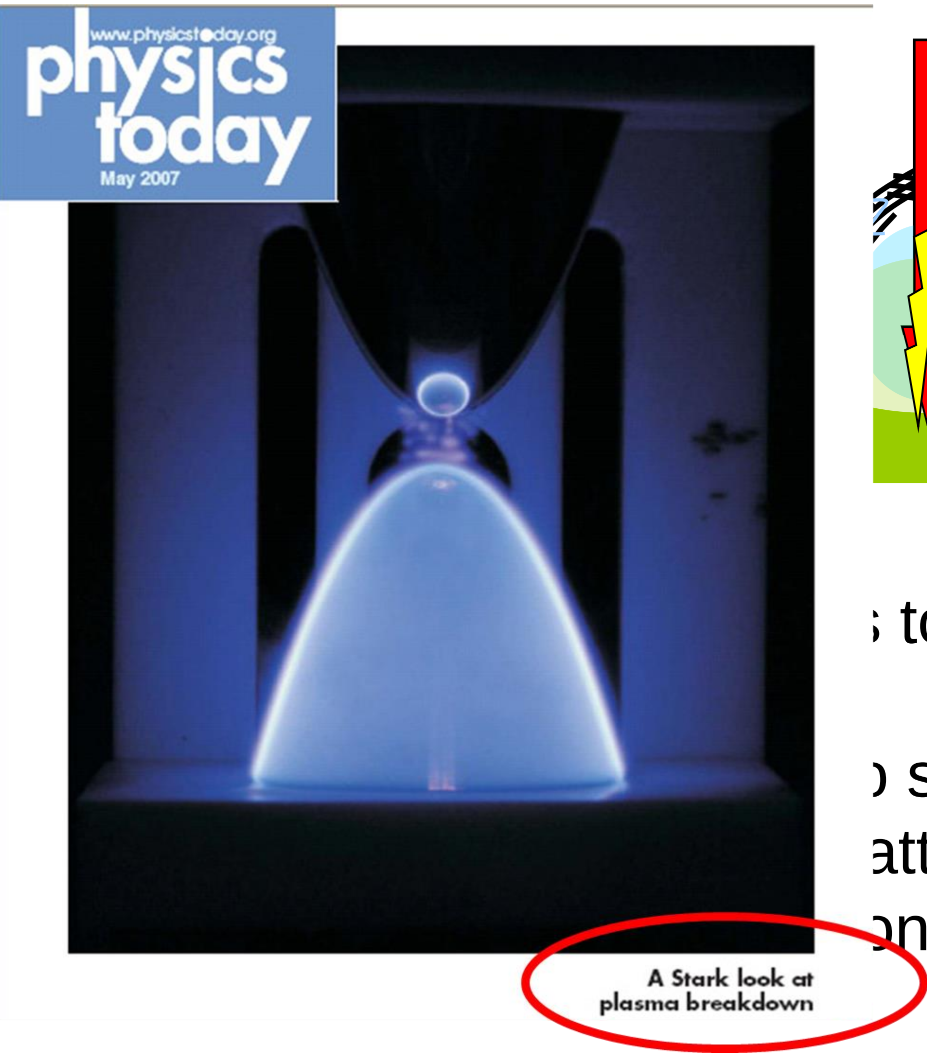
2) removal of samples mass (ablation)

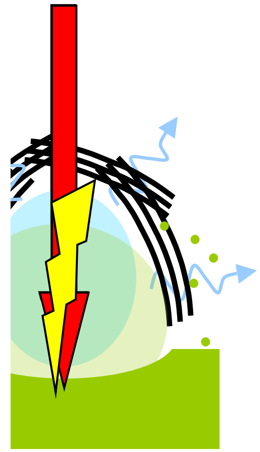






3) plasma formation (breakdown)





absorption of the laser radiation by the vapor

continuum emission

electrical breakdown and plasma formation

shock wave

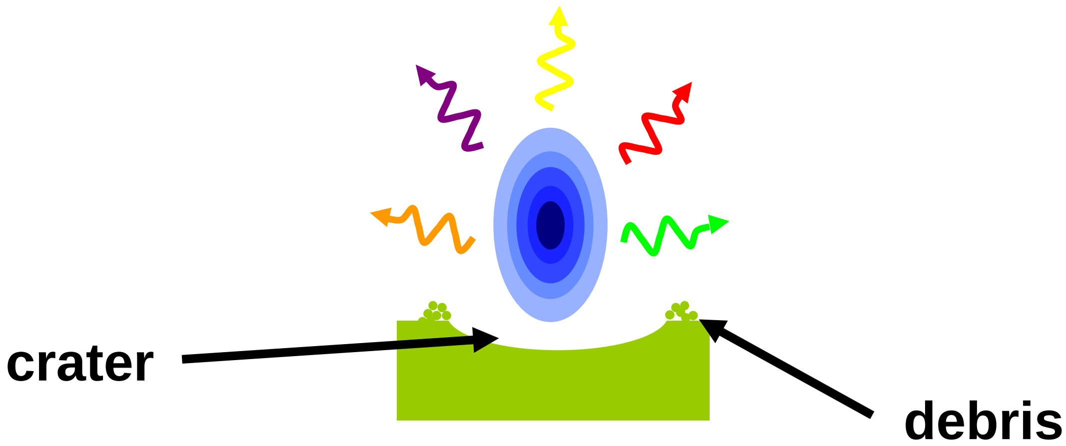
bremsstrahlung

; to illuminate the vapor plume.

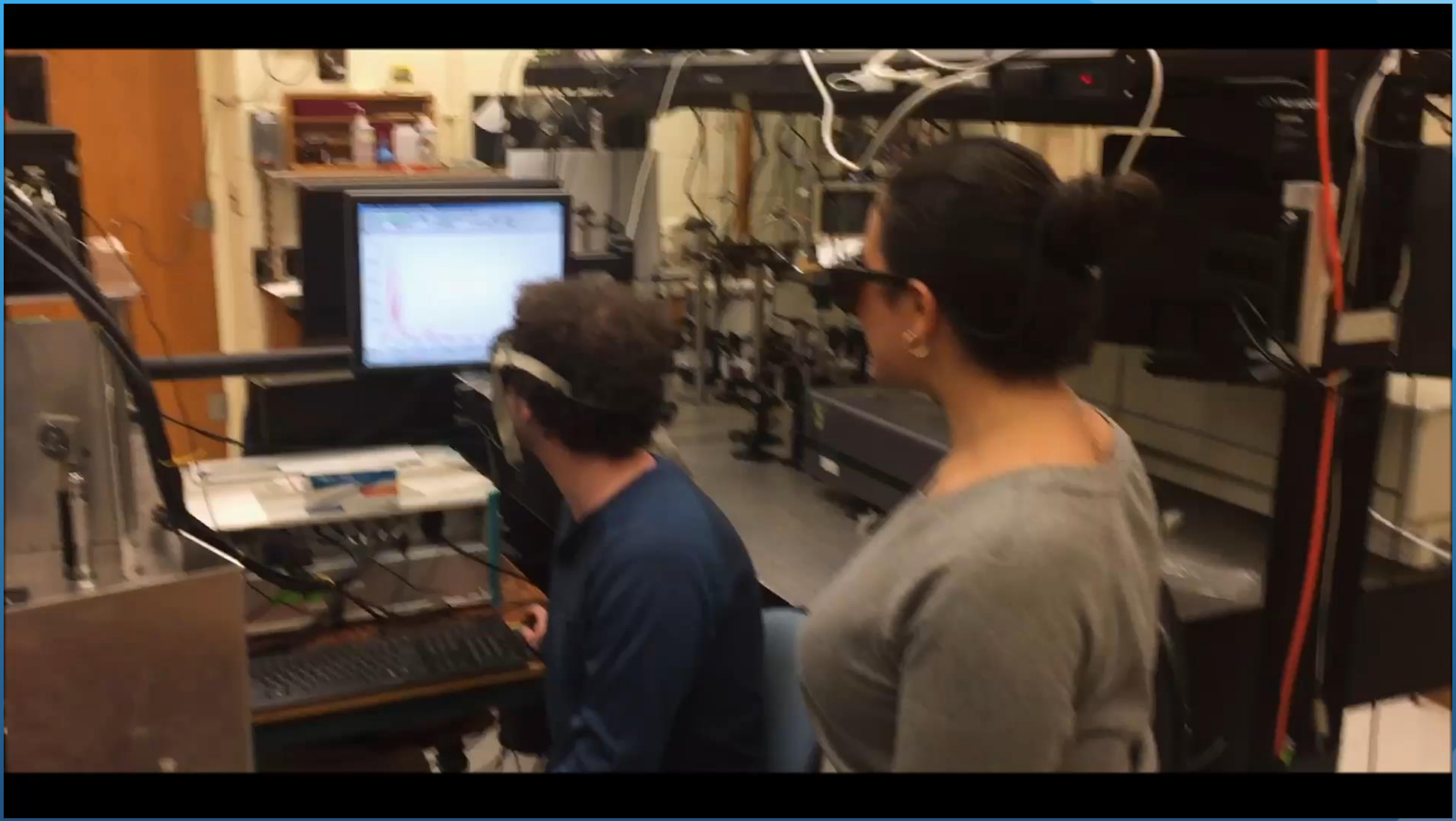
› sub-micrometer droplets that
attering of the laser beam,
›nization, and plasma formation.

4) expansion and element specific emission (atomic or ionic)

spontaneous emission as atoms/ions decay to ground state

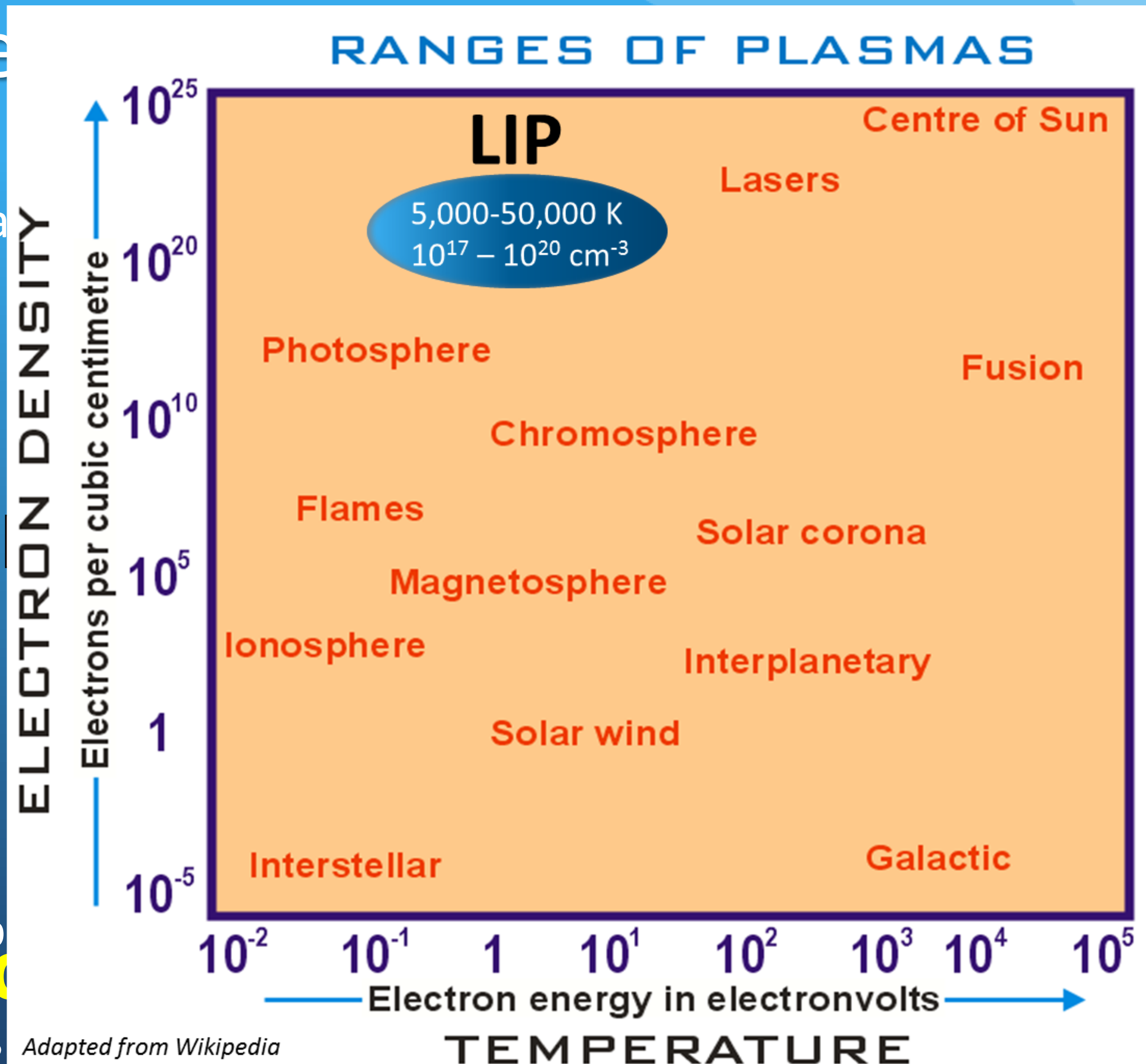


- The dynamic evolution of the plasma plume is then characterized by a fast expansion and subsequent cooling.
- Approximately 1 microsecond after the ablation pulse, spectroscopically narrow atomic/ionic emissions may be identified in the spectrum.



Lase

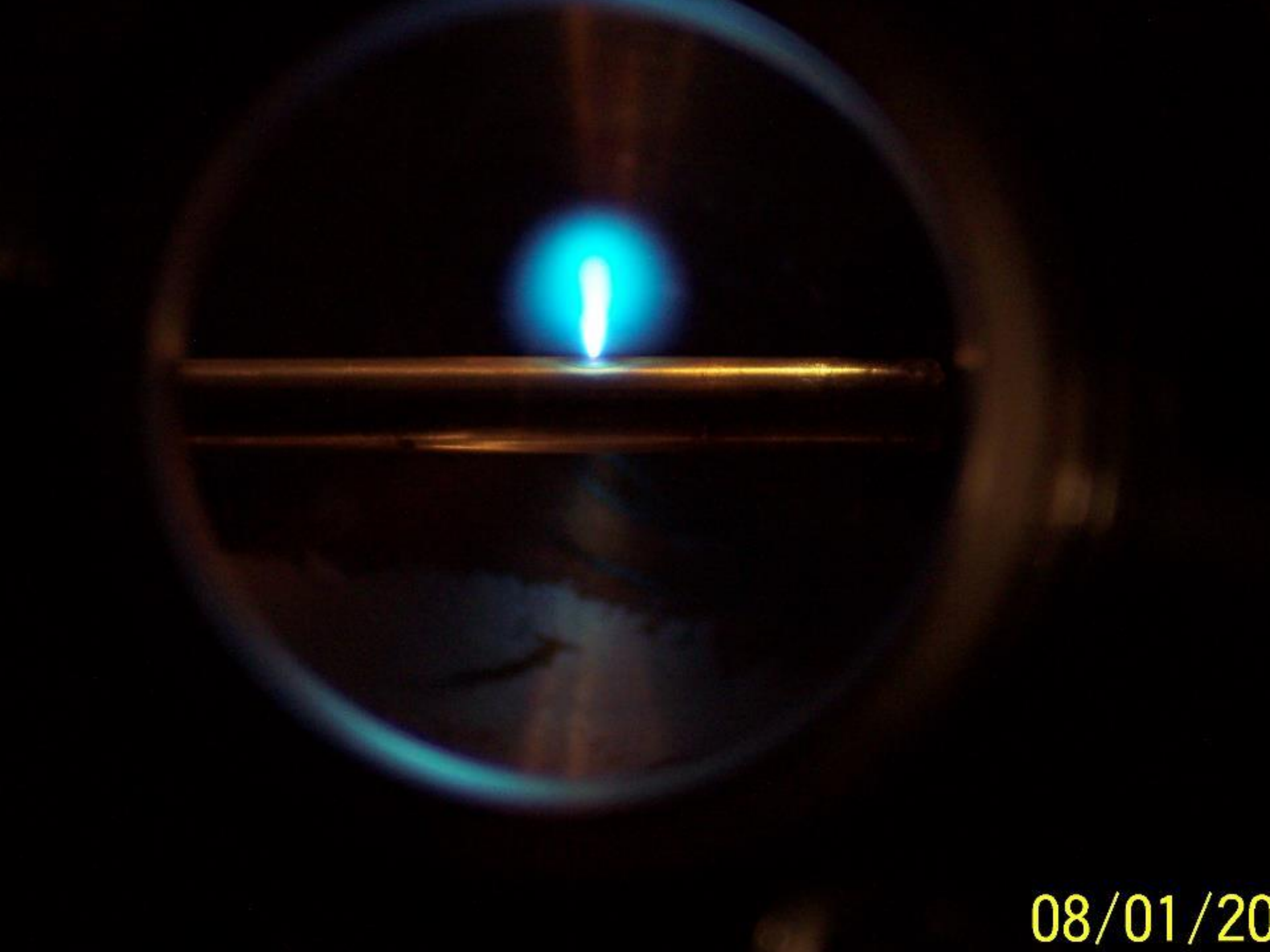
- Wha



- Can b

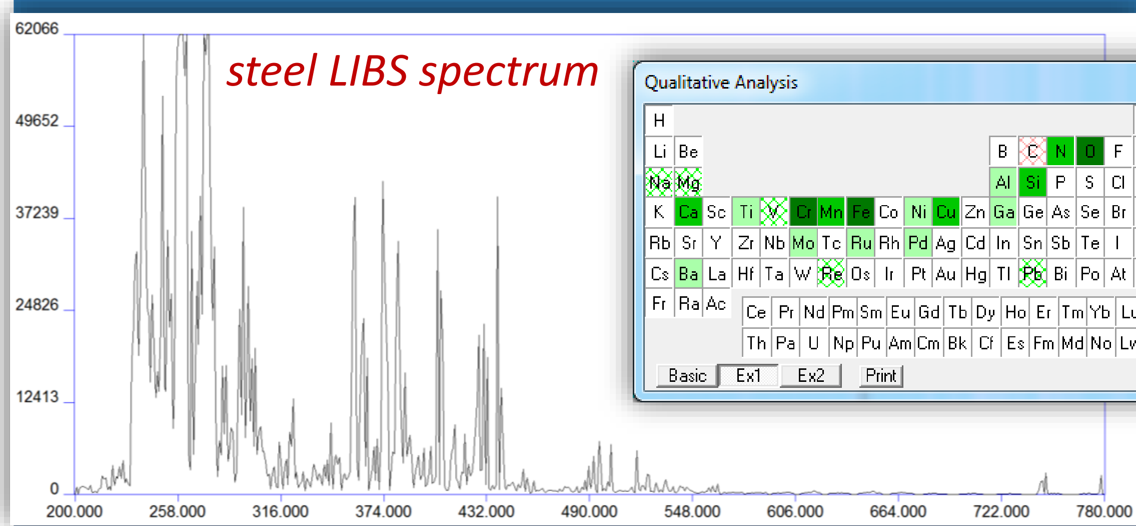
We c

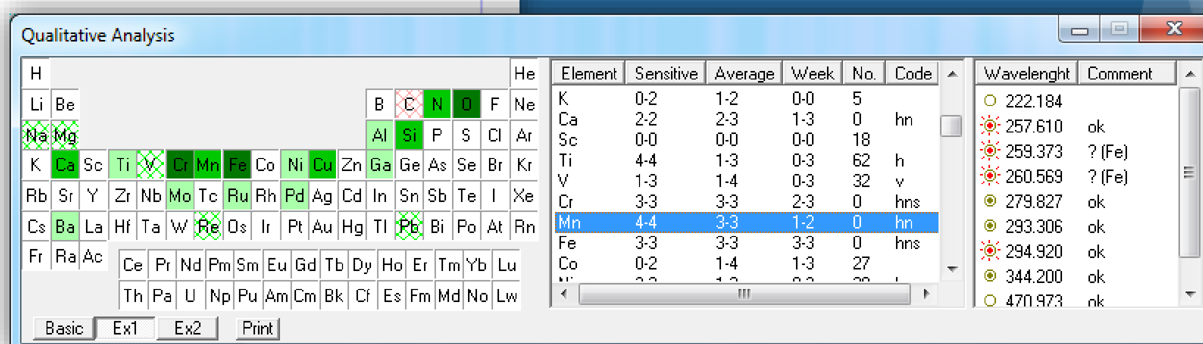
- Thres


08/01/20

The Goal of LIBS Plasma Creation

- to create an <u>optically thin plasma</u> which is in thermodynamic equilibrium (or LTE) and whose elemental composition is the same as that of the target/sample
  - if achieved, **atomic emission spectral line intensities** can be related to **relative concentrations** of elements (sometimes absolute concentrations)
  - typically these conditions are only met *approximately*.



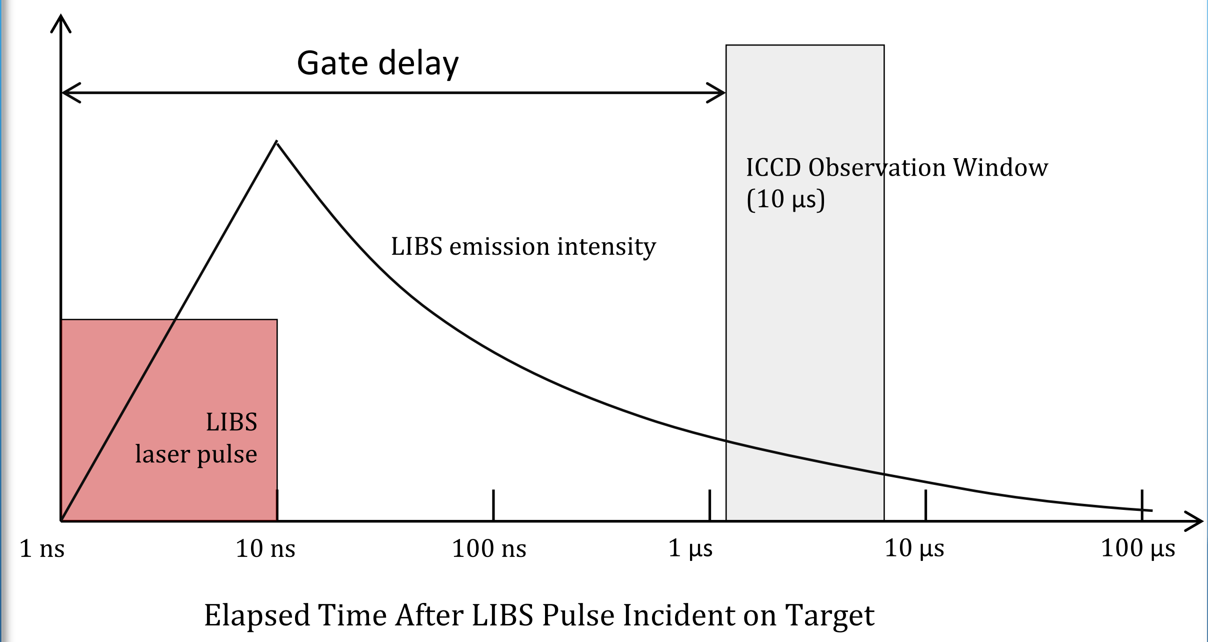


When we do a time-resolved spectroscopy of the plasma, we call it:

"Laser-induced breakdown spectroscopy"

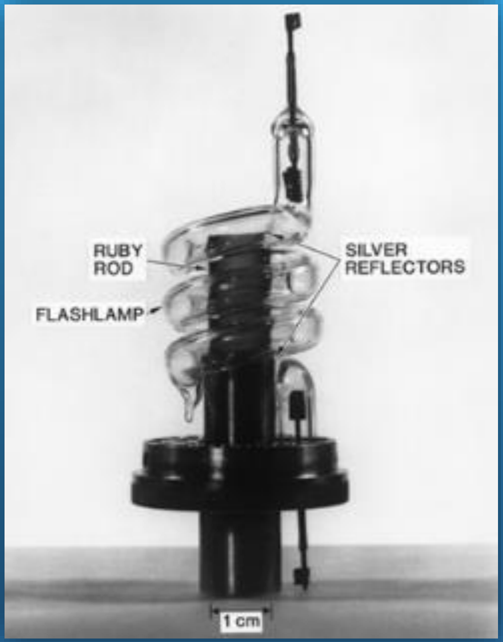
or

LIBS



1960

Maiman: first ruby laser



1962

Brech, Cross: Birth of LIBS: detection of spectrum from ruby laser-induced plasma

Spectrochemical analysis using a pulsed laser source

(*Received* 12 *July* 1963)

INTRODUCTION

SINCE the discovery of the optical maser, or laser, announced some three years ago, considerable scientific thought and effort have been expended toward making it a useful tool. In 1962, BRECH [1] used a ruby laser to produce vapors which were excited by an auxiliary spark source to analyze metallic and nonmetallic materials through their emission spark spectra. Early in 1963, we observed atomic emission spectra produced by the coincident vaporization and excitation of metals and nonmetals by means of a giant-pulse ruby laser. Now it can be shown that spectra produced solely by laser excitation exhibit fairly reproducible quantitative relationships among the various elemental constituents of the sample. And, for the first time, spectroscopists have a means of directly exciting solid materials without having to supply electrical power to the sample material. The sample need not be an electrical conductor, and it can be situated in an environment hostile to more conventional analytical techniques: for example, within a furnace or a radioactive environment.

The experiments to be described were designed to test this quantitative nature of pure laser excitation. Neither the details of the apparatus nor the type of sample is of great significance in itself. The precision of the data obtainable is the object of the experiment.

1964

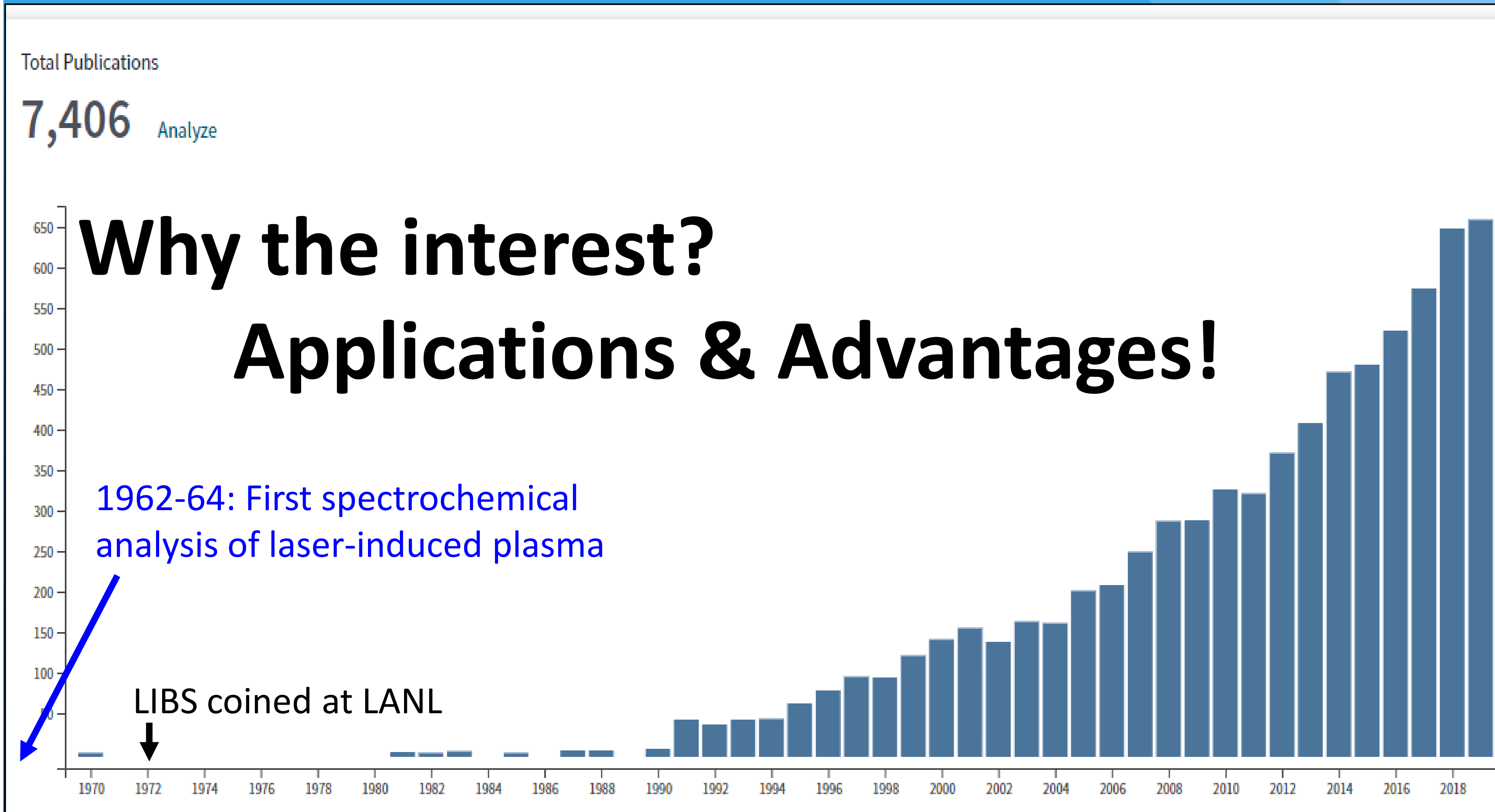
Runger et al. First direct spectro-chemical analysis by LIBS

1965

Zel'dovich, Raizer: First theoretical model for laser breakdown of a gas

History

"laser-induced breakdown spectroscopy" or "laser-induced plasma spectroscopy" @ Web of Science (Thomson Reuters)



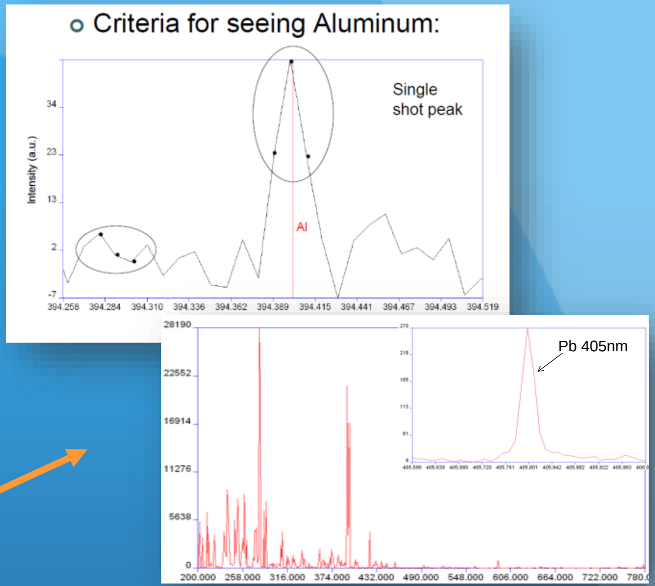
Why the interest?

Applications & Advantages!

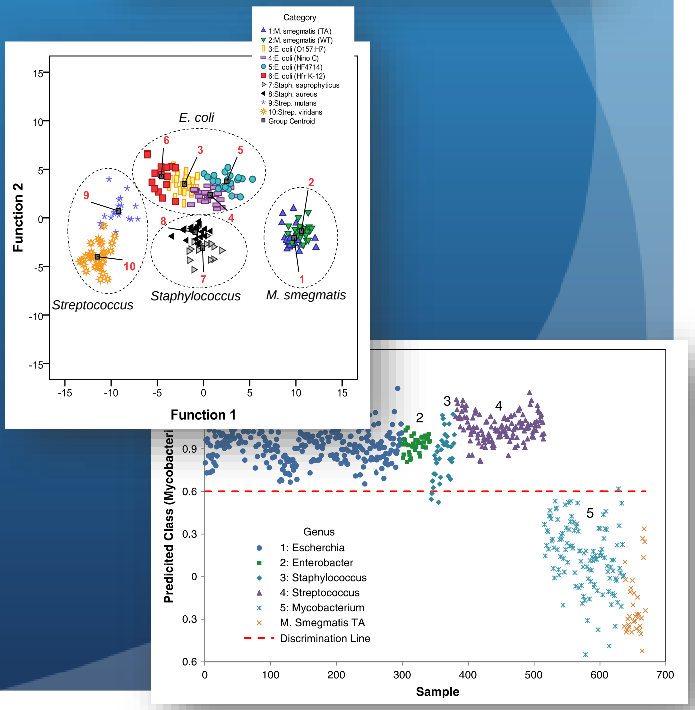
Applications of LIBS

No matter what your application is, <u>you will be doing one of two things:</u>

1. Attempting to quantify the amount/concentration of some element by analyzing peak intensities



2. Attempting to identify a target based on its unique elemental composition by analyzing the presence intensity of all/many lines



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Advantages of LIBS – multi-element sensitive

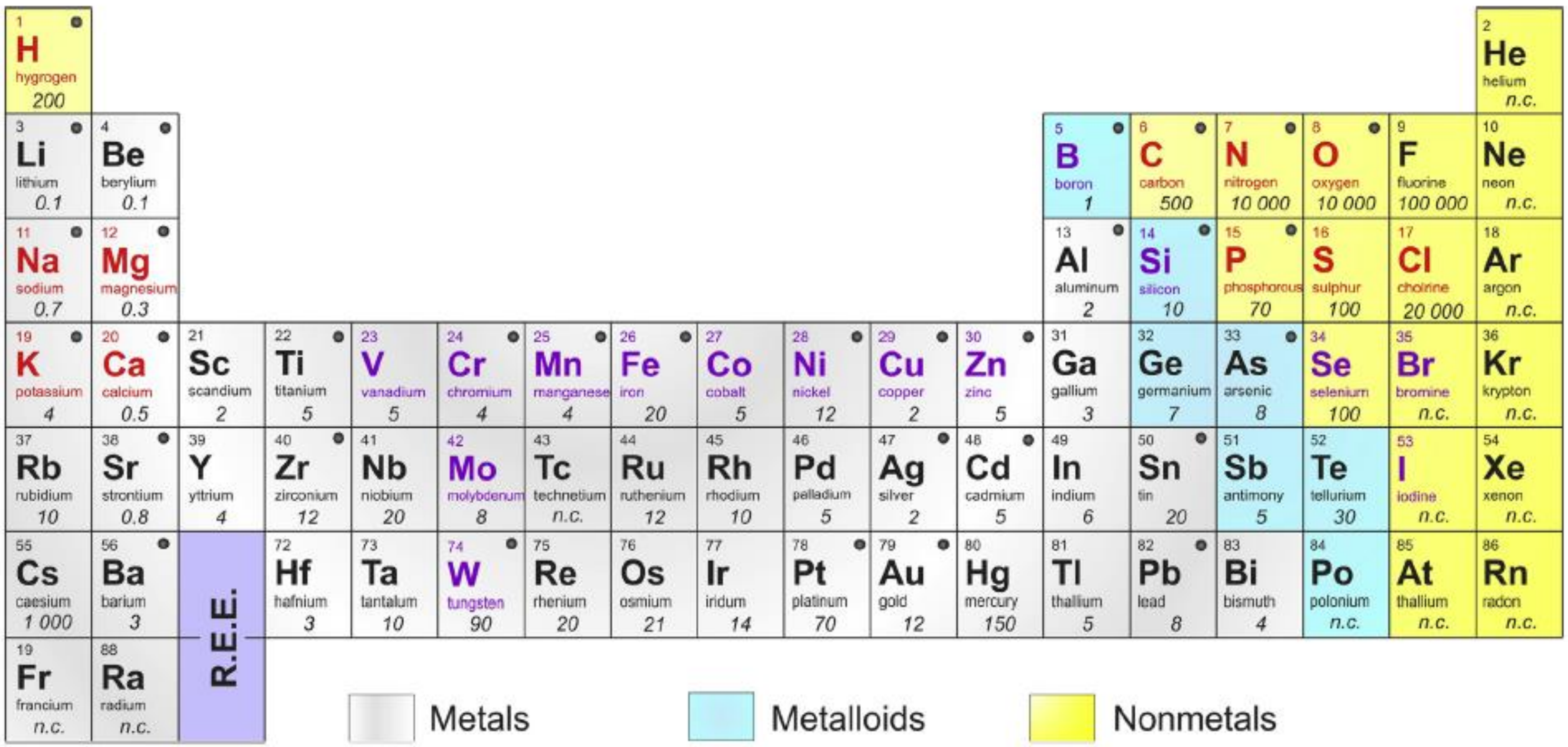


Fig. 1. Periodic table of the elements and LIBS analysis. Almost all elements, including metals, are detectable within biological tissues via LIBS. The essential chemical elements for most living organisms are displayed as follows: bulk biological elements are in red and essential trace inorganic elements for plants or animals are in purple, according to [25]. Endogenous and exogenous elements already detected in tissues via LIBS in previous biological studies are marked with a black dot. The theoretical LOD is given in parts per million and is indicated by the number in italics under the chemical name of the element. R.E.E.: rare earth elements.

Review

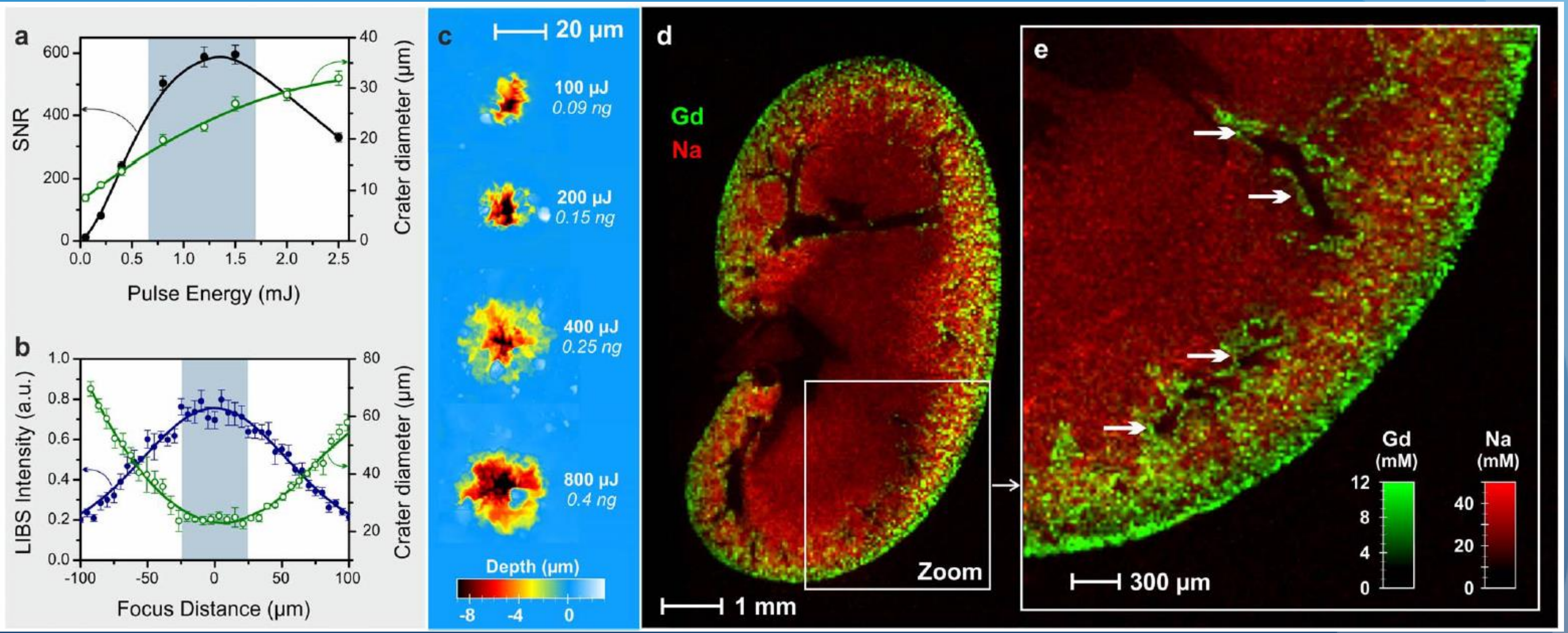
Elemental imaging using laser-induced breakdown spectroscopy: A new and promising approach for biological and medical applications

Benoit Busser[a,b,c,*], Samuel Moncayo[b], Jean-Luc Coll[a], Lucie Sancey[a,1], Vincent Motto-Ros[b,1]

Coordination Chemistry Reviews 358 (2018) 70–79

Advantages of LIBS - spatial resolution

- Laser allows point sampling (1-100 micron)
- Elemental "surface maps" can then be created



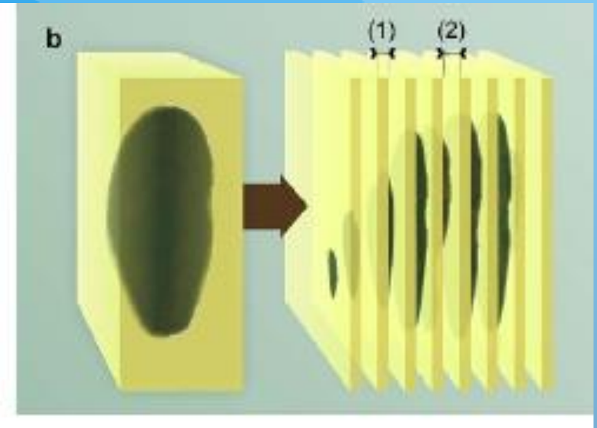
Laser spectrometry for multi-elemental imaging of biological tissues

L. Sancey*, V. Motto-Ros*, B. Busser, S. Kotb, J. M. Benoit, A. Piednoir, F. Lux, O. Tillement, G. Panczer & J. Yu

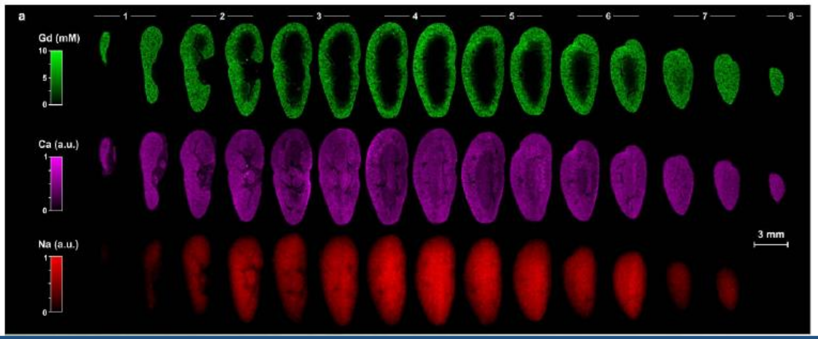
SCIENTIFIC REPORTS | 4 : 6065 | DOI: 10.1038/srep06065

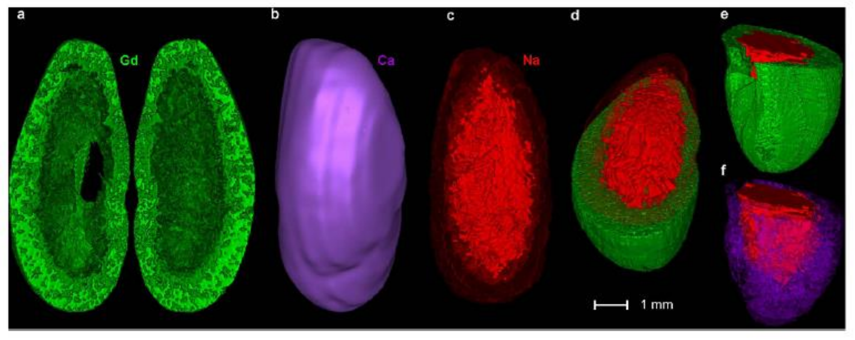
Advantages of LIBS - depth profiling

- Because laser only removes µg to ng of material, ablation crater only microns deep



- Subsequent shots thus sample progressively deeper layers





3D Imaging of Nanoparticle Distribution in Biological Tissue by Laser-Induced Breakdown Spectroscopy

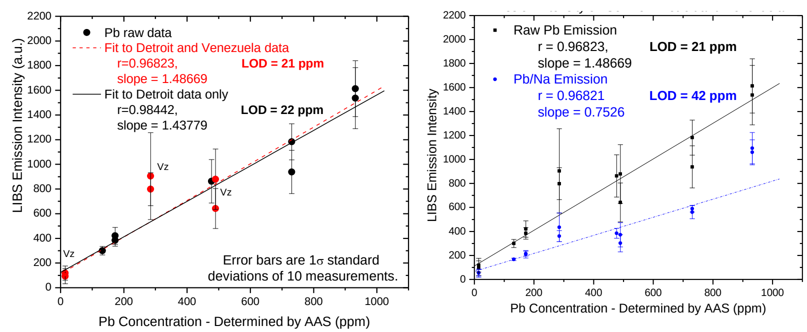
Y. Gimenez[1], B. Busser[1], F. Trichard[1], A. Kulesza[1], J. M. Laurent[2], V. Zaun[3], F. Lux[1], J. M. Benoit[1], G. Panczer[1], P. Dugourd[1], O. Tillement[1], F. Pelascini[3], L. Sancey[1] & V. Motto-Ros[1]

SCIENTIFIC REPORTS | 6:29936 | DOI: 10.1038/srep29936

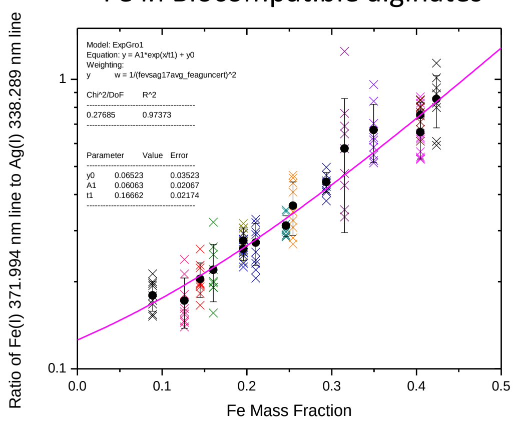
Advantages of LIBS – sensitivity & speed

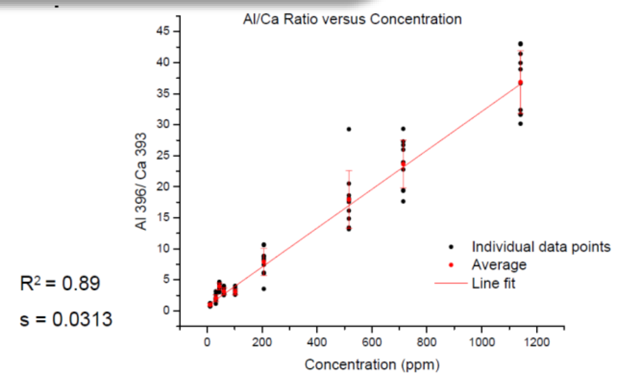
- Concentrations of 1-100 ppm usually detectable in seconds using a standard LIBS apparatus

Lead in Soil (Detroit and Venezuela)



Fe in Biocompatible alginates





Advantages of LIBS - portability / standoff

- Apparatus is compact, low weight; can be made man-portable
- All optical technique, so can be done at a distance "stand-off"

First responder CBRNE prototypes have been built…

Backpack contai
broadband high-
resolution
spectrometer, las
power supply,
computer, and ba

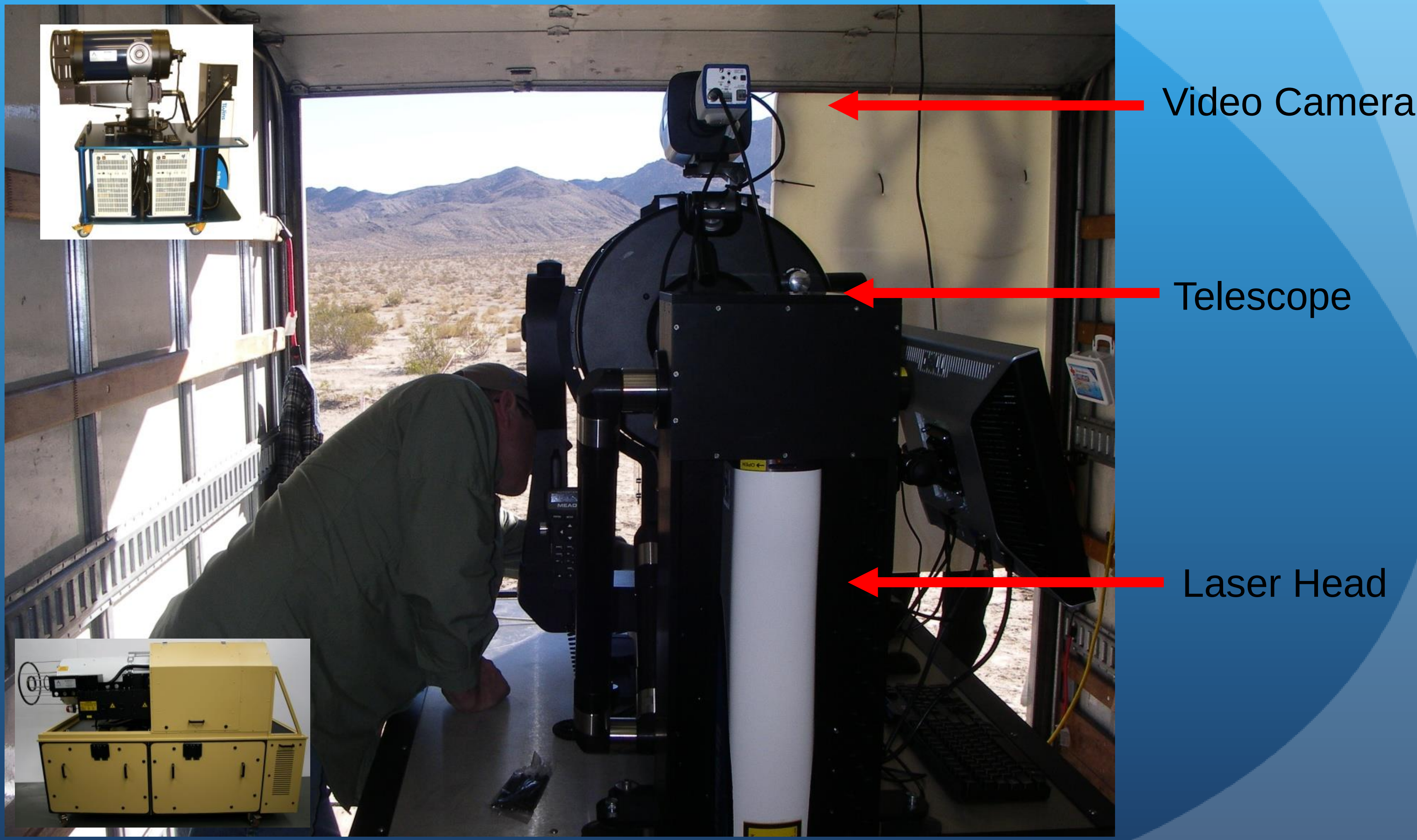
ad's-up display

nd-held probe contains
er, joystick for control,
l focus optics

Microplasma/
LIBS Event

courtesy of Ocean Optics.

High-energy remote systems have been built…



Courtesy of A.J. Miziolek, A. Whitehouse

Commercial benchtop systems have been built…

J200 – Applied Spectra

ChemReveal LIBS Desktop Elemental Analyzer – TSO

Hand-held systems have been built…

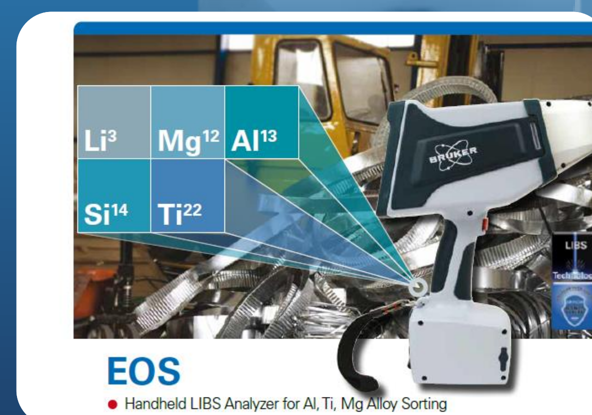
mPulse – Oxford Instruments

LIBZ – SciApps, Inc

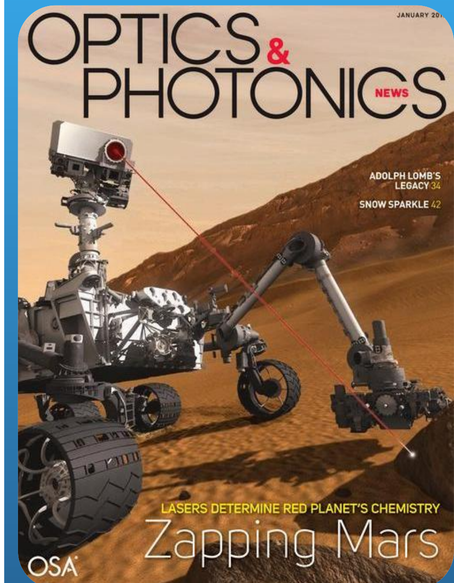
NanoLIBS – B&WTek

ChemLite- TSI, Inc

EOS500 - Bruker



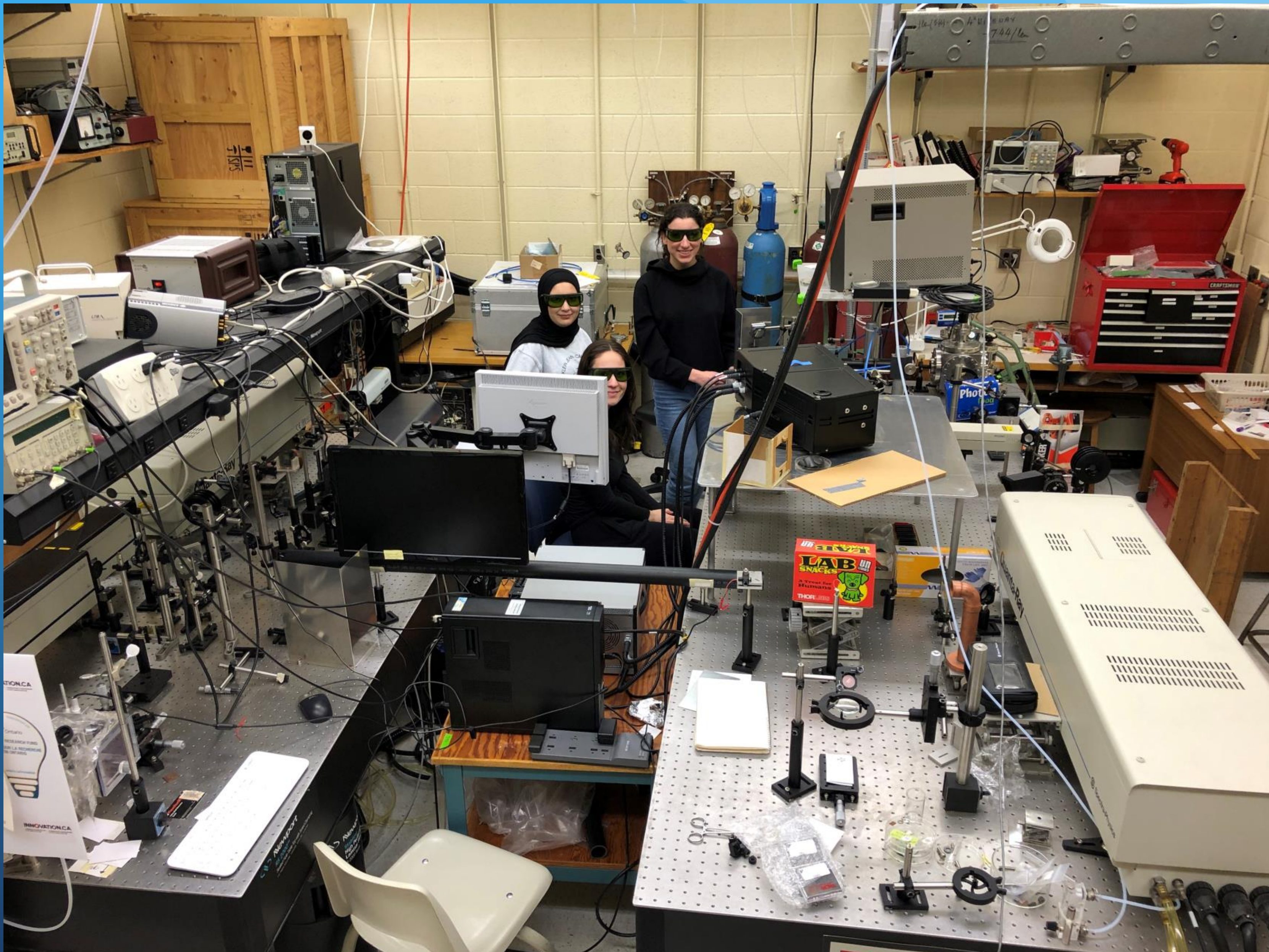
And a system has gone to Mars…

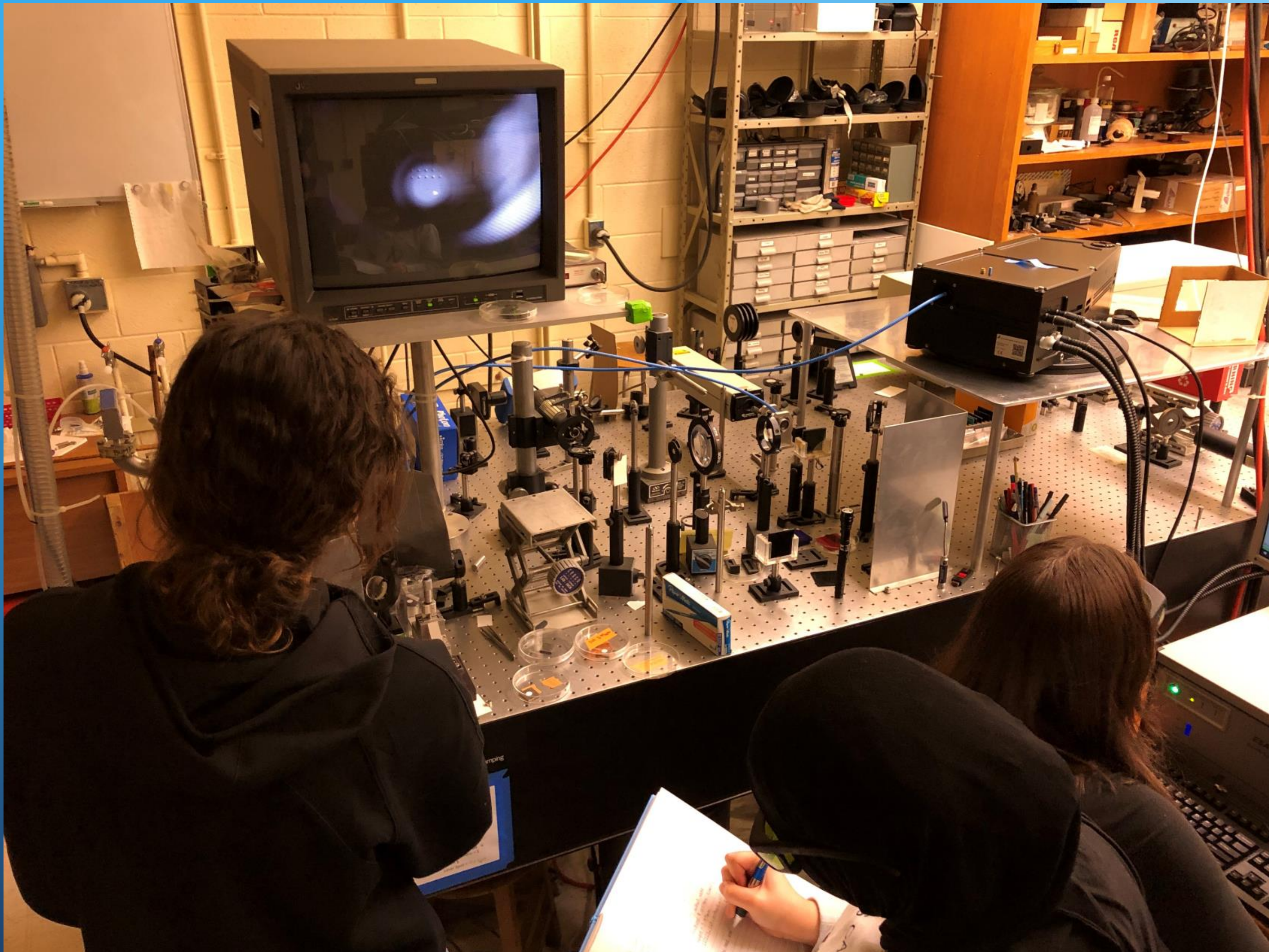


And a system has gone to Mars…

Los Alamos
NATIONAL LABORATORY

And a system has gone to Mars…


LAB SNACKS
A Treat for Humans
INNOVATION.CA



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Windsor Star Digital

THE WINDSOR STAR DIGITAL

THE WINDSOR STAR

THE WINDSOR STAR

What are you looking for this afternoon? Search

9°C

March 29, 2013

NEWS / Police And Fire

Suspicious powder at National Bank not dangerous, police say

ShareThis

Firefighters and hazardous material specialists gather on Pitt Street West in response to a report of a suspicious white powder at the Canada Post building on Ouellette Avenue in Windsor, Ont. on April 18, 2012. (Nick Brancaccio / The Windsor Star)

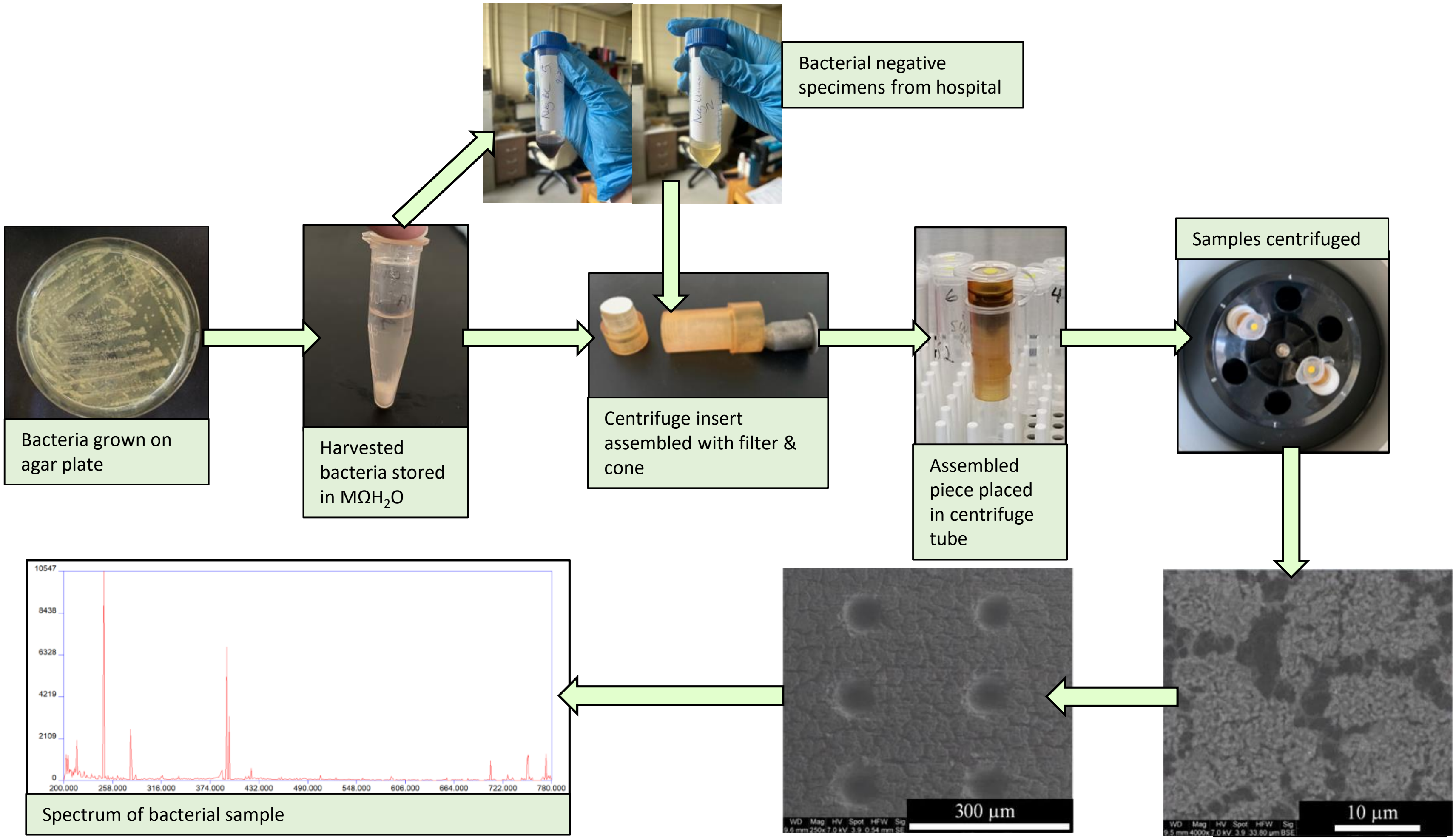
A hazardo
Windsor
fire and ambulance personnel des
inside a pencil case that had beer
after 4 p.m., two firefighters donnec
workers sifted through the found obj
origin or makeup of the powder. It has been taken to a laboratory in Etobicoke for testing.

So why?

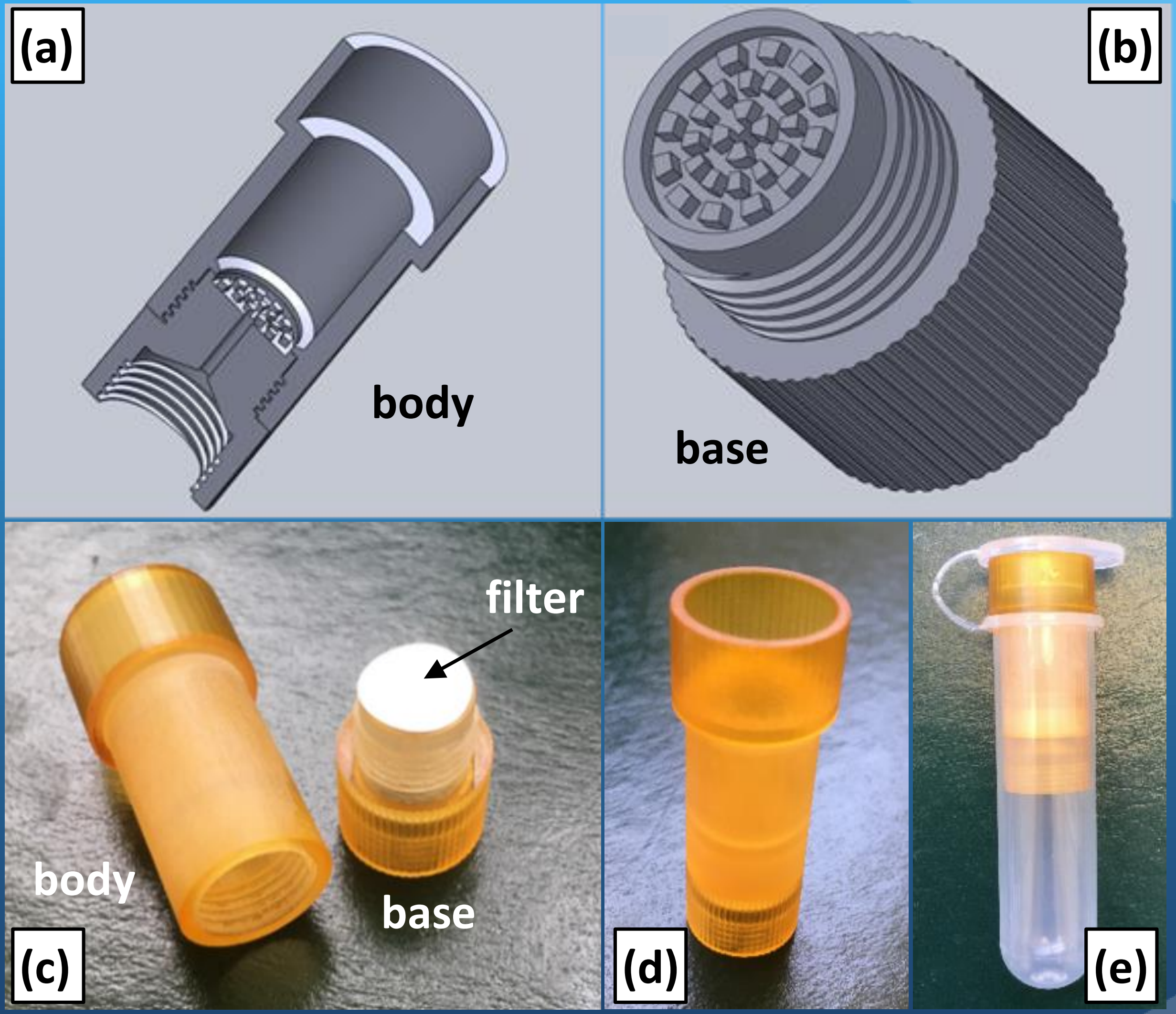
"It is well-accepted that the microbiological expertise and cost required to perform these identifications preclude their common use as a screening mechanism to prevent human infection."[1]

[1]Tarr, P.I. 1995. *Escherichia coli* O157:H7: clinical, diagnostic, and epidemiological aspects of human infection. Clin. Infect. Dis. 20, 1-8.

Methodology – Bacterial Growth & Sample Prep



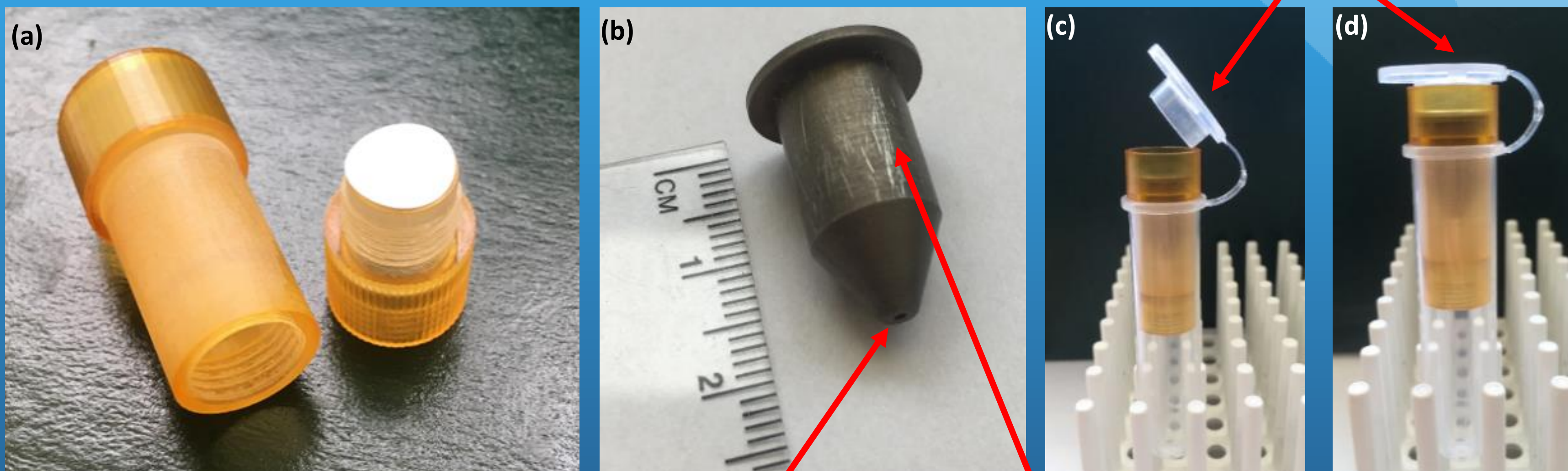
The Centrifuge Insert



Concentrating Bacteria With a Cone

Centrifuge tube cap presses cone into filter

19 mm long Al cone

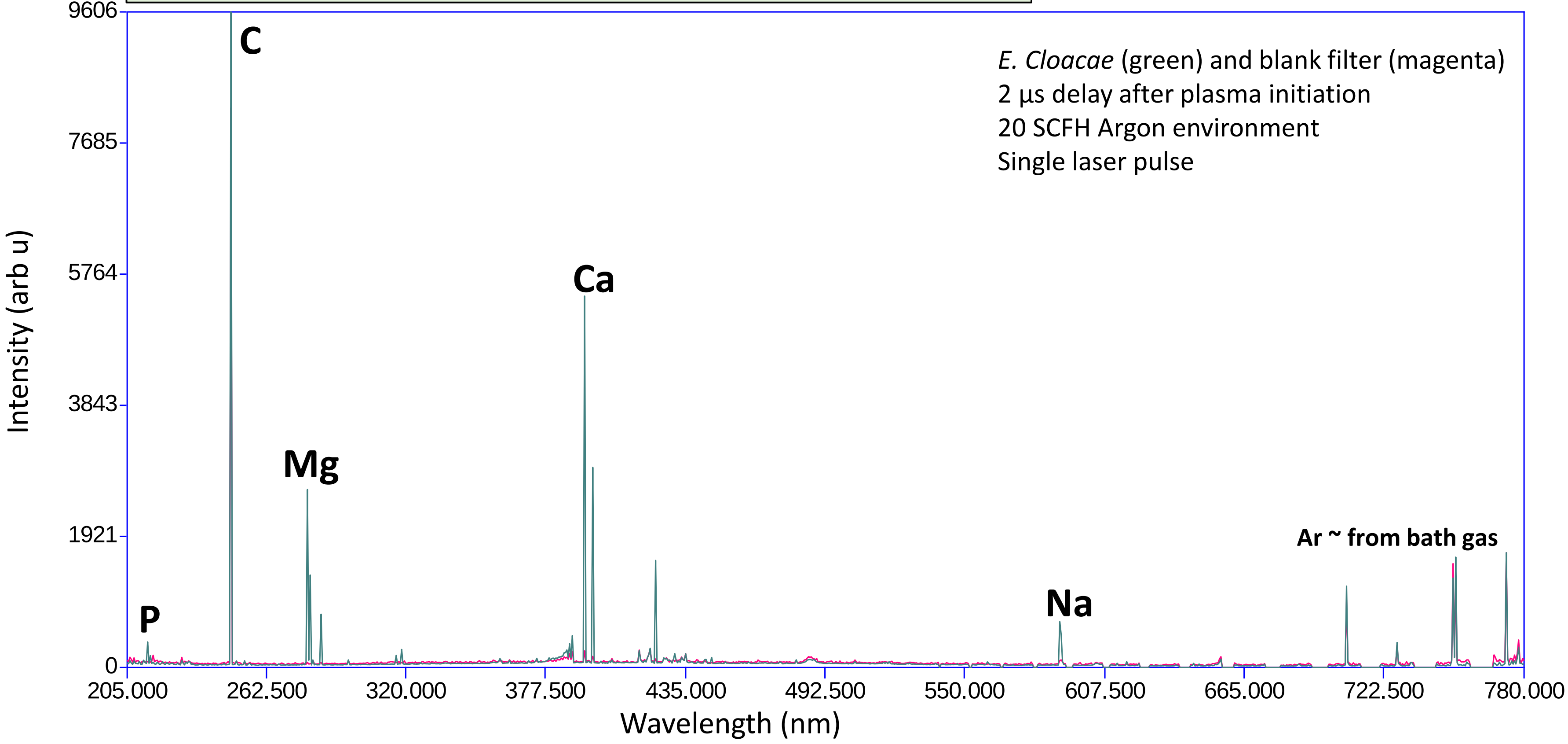


1 mm hole at apex

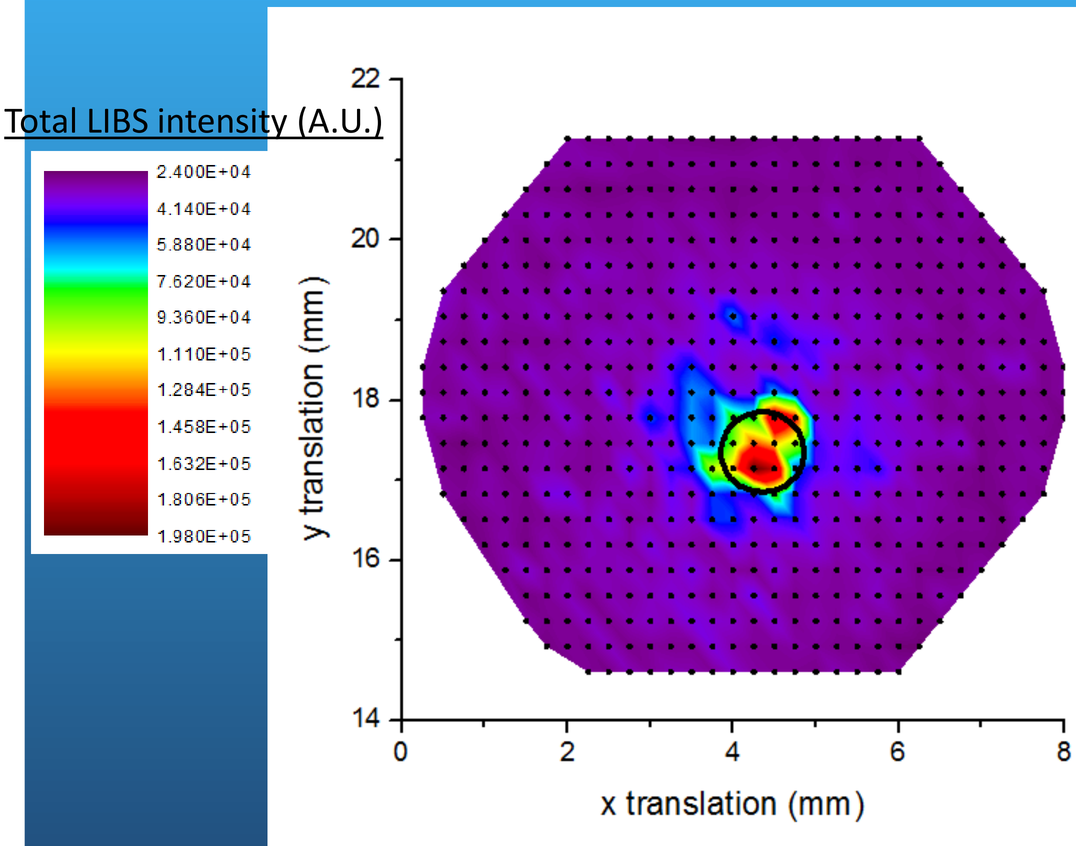
Holds 1 mL of fluid

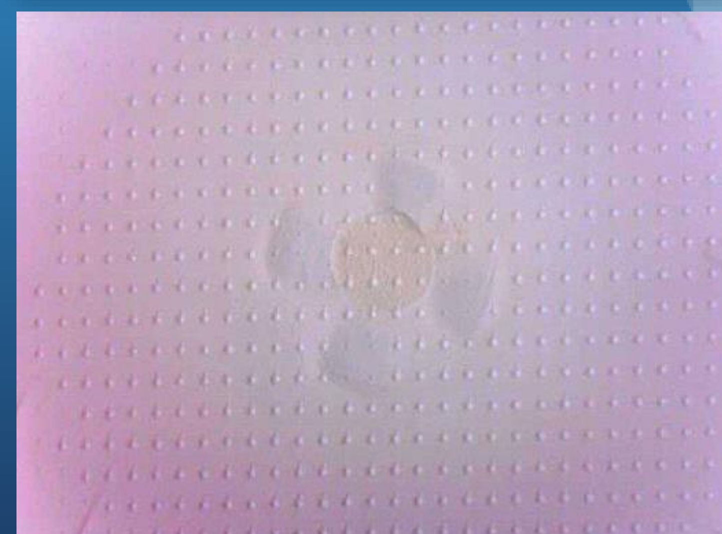
Cone vertex press fit into filter

After laser ablation, light from the plasma is dispersed which gives the elemental composition

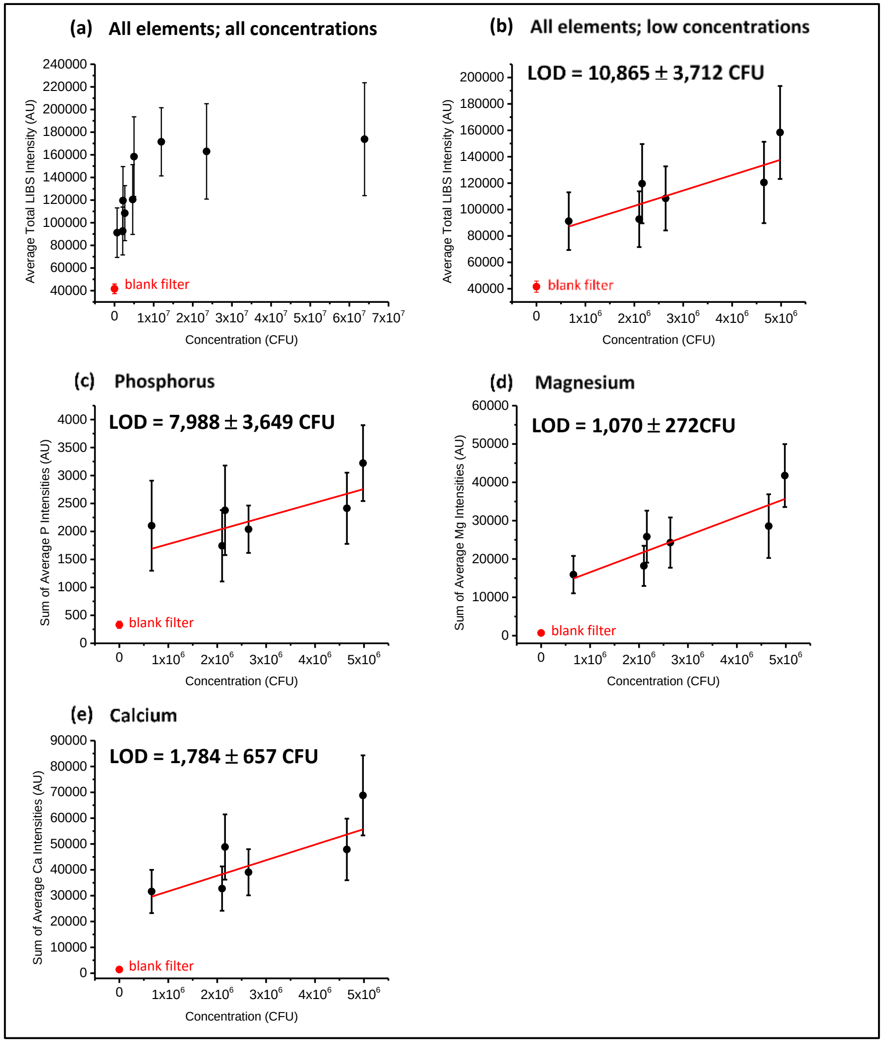


Shooting Bacteria Concentrated With Cone





Limits of Detection



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.

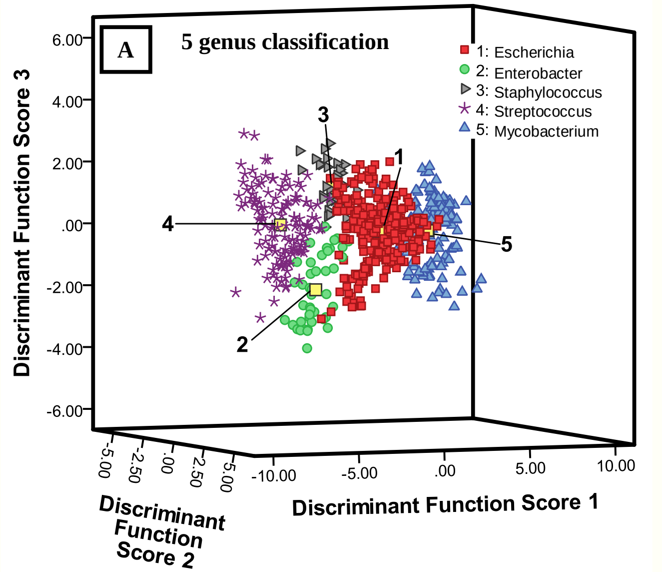
LOD's calculated using only certain elements observed in the LIBS spectra and present in very low concentrations in the filter were even lower:

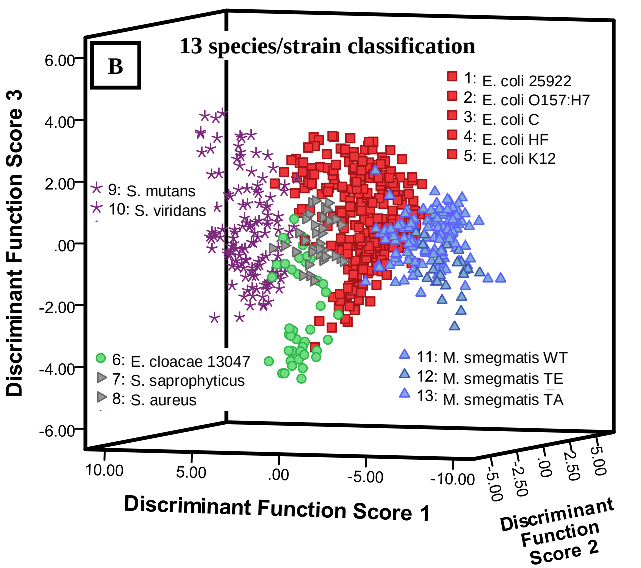
1,070 ± 272CFU for magnesium
1,784 ± 657 CFU for calcium.

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

When performed with no background

- ✓ 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 %

Detecting Bacteria in Water by Reducing Scatter per Filter

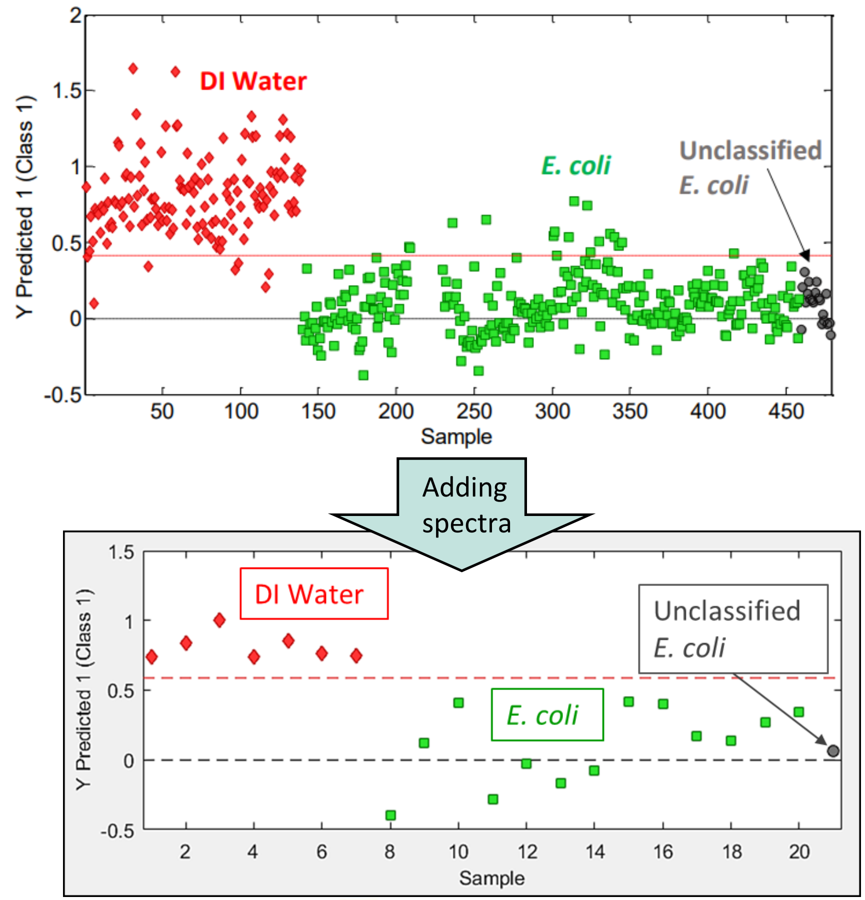
- Adding spectra together (in Excel) to produce **1 measurement per filter**

✓ Can reliably detect bacteria in DI water using 'addall' method

✓ Same method works with ultrapure water

Bacterial library:

→ 164 independent variables created from 19 lines and the ratios of these 19 lines
→ ~600 spectra
→ **Sensitivity: 100%**
→ **Specificity: 100%**



Diagnosing Species with Machine Learning

DFA on Ratio Model

ANN on Ratio Model

PCA-ANN on Ratio Model

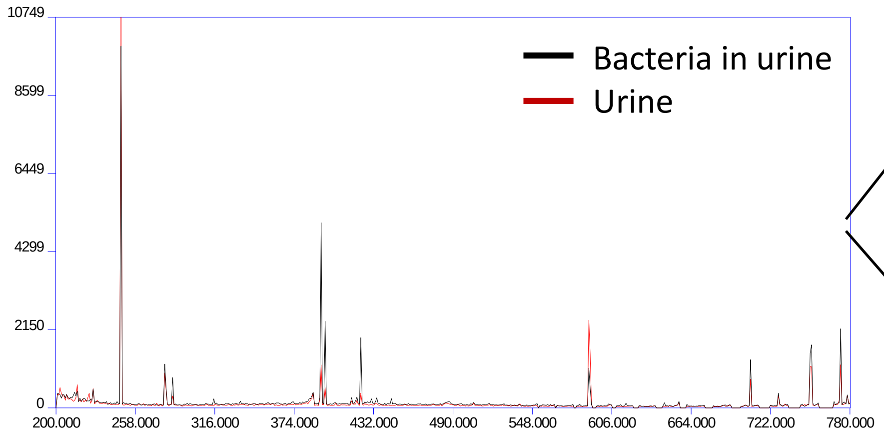
PCA-ANN on Full Spectrum Data*

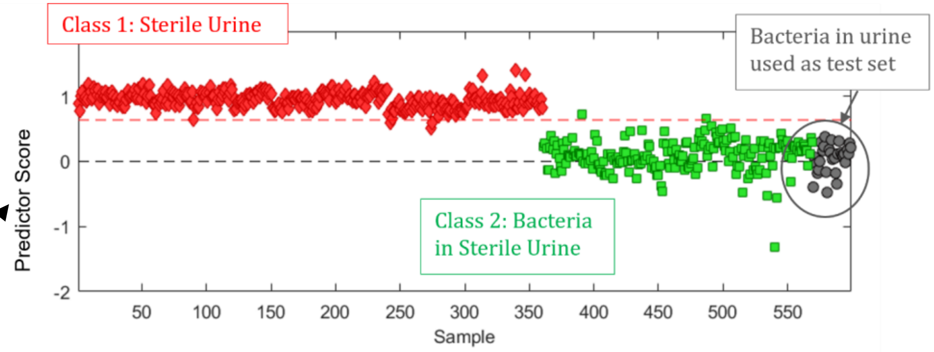
- Started using the "whole spectrum" from 200 nm - 590 nm. 42,000 variables.
- Perform unsupervised PCA first (implemented in Python), reduce to 10 PC's. (~30 minutes).
- Models are trained on 80% of single shot data, 20% reserved for testing. (~15 seconds).

PCA-ANN With Full Spectrum Data			
	E. coli	S. aureus	E. cloacae
Sensitivity	98.04 %	93.27 %	91.23 %
Specificity	97.71 %	97.22 %	96.12 %
Classification Error	2.13 %	4.28 %	6.33 %

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

Diagnosing Bacteria in Urine





PCA-ANN With Full Spectrum Data*			
	S. aureus	E. coli	E. cloacae
Sensitivity	100 %	100 %	91.67 %
Specificity	100 %	95.83 %	100 %
Classification Error	0.00 %	2.09 %	4.17 %

*classification using 80:20 split

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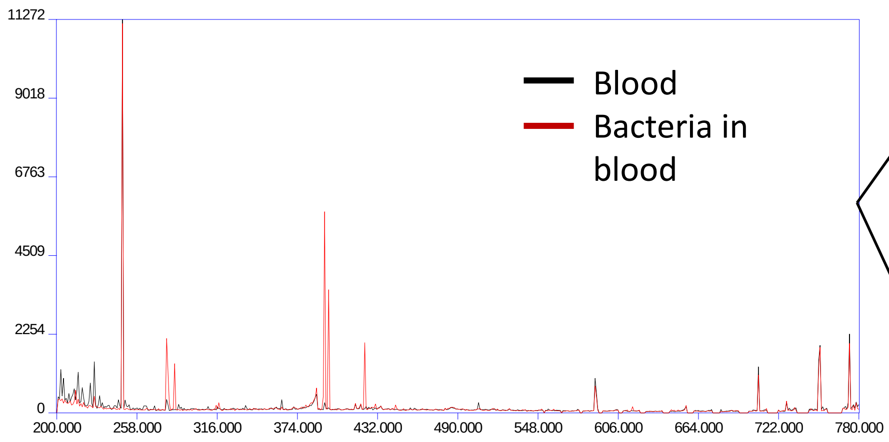
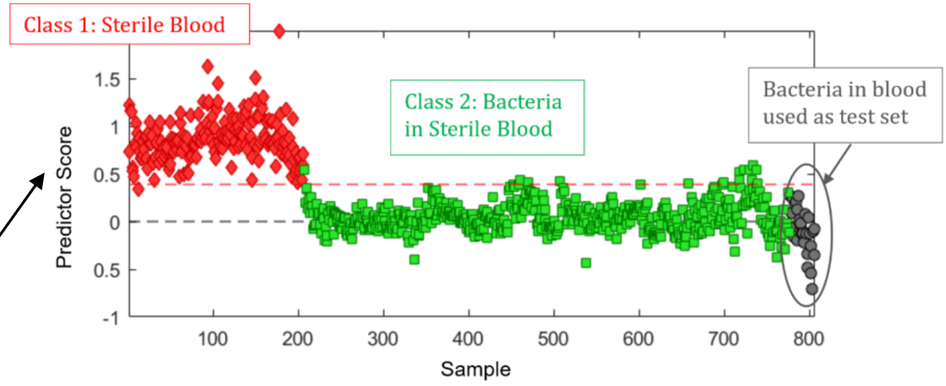
Diagnosing Bacteria in Blood





PCA-ANN With Full Spectrum Data*				
	S. aureus	E. coli	E. cloacae	P. aeruginosa
Sensitivity	100 %	100 %	100 %	100 %
Specificity	100 %	100 %	100 %	100 %
Classification Error	0.00 %	0.00 %	0.00 %	0.00 %

*classification using 80:20 split

External Validation of PCA-ANN in Blood and Urine

- External validation done in both urine and blood
- Improvements need to be made on external validation

Urine	*E. coli*	*S. aureus*	*E. cloacae*	
Average Sensitivity	75.83 %	90.00 %	66.67 %	
Blood	***E. coli***	***S. aureus***	***E. cloacae***	***P. aeruginosa***
Average Sensitivity	80.67 %	65.33 %	92.67 %	92.50 %

Urine

E. coli	Predicted			
Sample #	*S. aureus*	*E. coli*	*E. cloacae*	Sensitivity
1	0	28	2	93.333333
2	2	8	20	26.666667
3	0	25	5	83.333333
4	0	30	0	100
Sum	2	91	27	75.833333

Blood

S. aureus	Predicted				
Sample #	*S. aureus*	*E. coli*	*E. cloacae*	*P. aeruginosa*	Sensitivity
1	30	0	0	0	1
2	1	0	29	0	0.0333333
3	30	0	0	0	1
4	30	0	0	0	1
5	7	23	0	29	0.2333333
Sum	98	23	29	0	0.6533333

Outline

1. Introduction of the Method. Laser-induced breakdown spectroscopy (LIBS)

2. Advantages of LIBS

3. Biomedical Applications of LIBS: A new paradigm for rapid pathogen identification

4. Concluding Thoughts

Conclusions

- LIBS provides an accurate, fast, spatially resolved, remote spectrochemical analysis of almost any type of target (solid, liquid, gas, powder)
- High degree of versatility and robustness suggests its adoption in many different interdisciplinary fields – including microbiology and medicine
- Experiments utilizing LIBS involve an exciting mixture of physics, laser science, and analytic chemistry (at a minimum)

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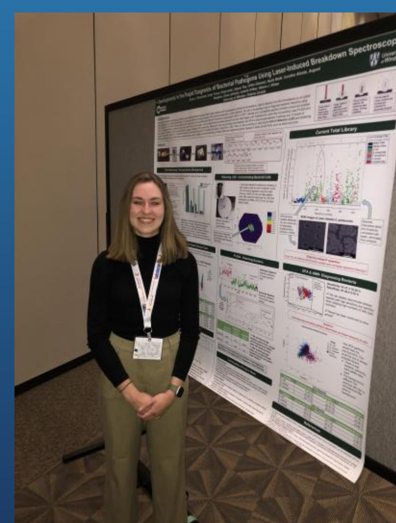
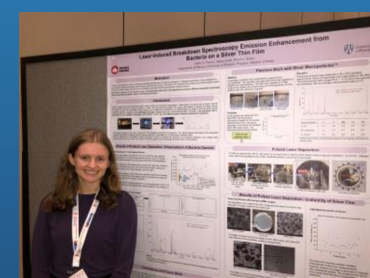
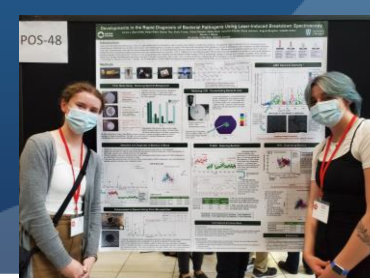
- ***University of Windsor*** Faculty of Science

The people who did the work…







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Thank you!