

Laser-Induced Breakdown Spectroscopy as a Tool for Rapid Elemental Bioanalysis



Eastern Analytical
Symposium and Exhibition

Princeton NJ November 12-14 2018

Analytical Techniques for Elemental Analysis of Solids (Dr. Lydia Breckenridge, presiding)

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Staph. epidermidis



University of Windsor

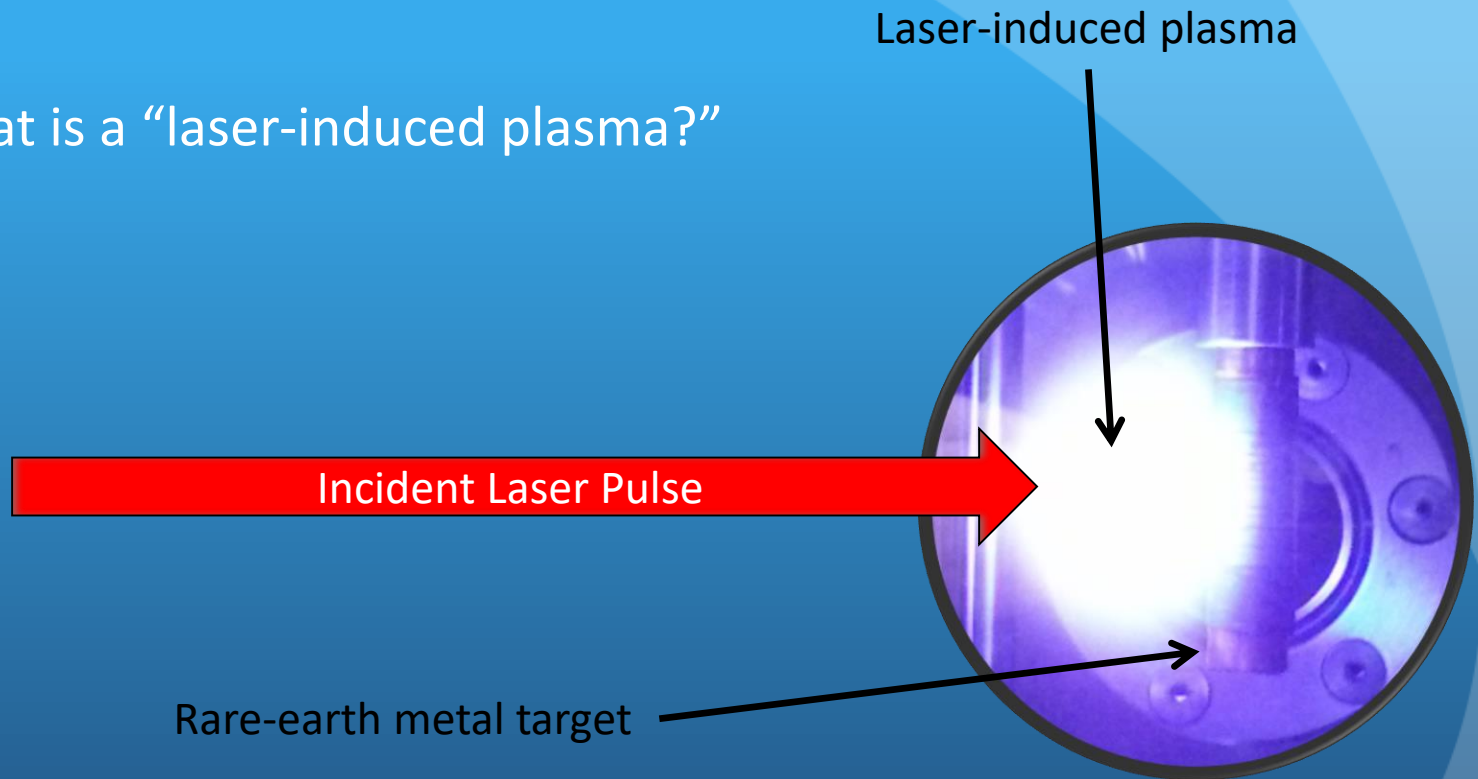
Windsor, Ontario, Canada

Outline

1. Introduction of the Method. Laser-induced breakdown spectroscopy (LIBS)
2. Comparison of LIBS with other analytic methods
3. Biomedical Applications of LIBS
 - a. A new paradigm for rapid pathogen identification
 - b. A real time assay for nutritional zinc deficiency
 - c. An ecological tool for analyzing fish otoliths
4. Concluding Thoughts

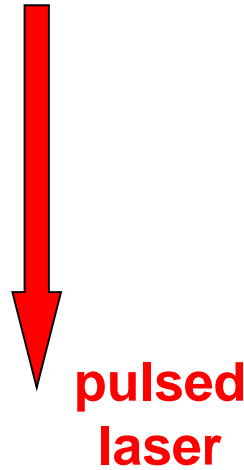
Laser-Induced Plasmas

- What is a “laser-induced plasma?”



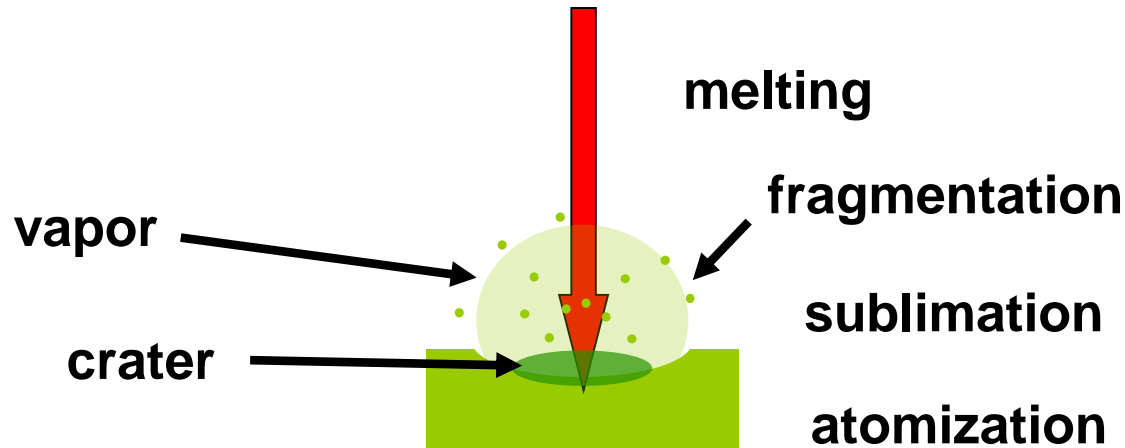
- Can be done with ns, ps, or fs lasers
- Threshold irradiance: $\sim 10^{10} - 10^{11}$ W/cm
(typically 1-100 mJ/pulse)

**1) laser interaction
with the target**



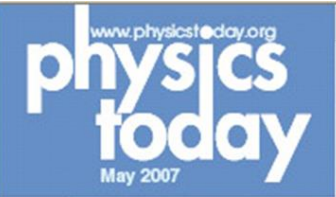
- initiated by absorption of energy by the target from a pulsed radiation field.
- pulse durations are on the order of nanoseconds, but can be performed with pico- and femto-second laser pulses.

2) removal of samples mass (ablation)

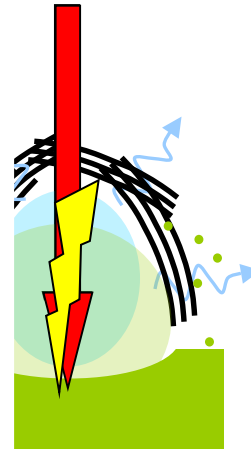


- absorbed energy is rapidly converted into heating, resulting in vaporization of the sample (ablation) when the temperature reaches the boiling point of the material.
- removal of particulate matter from the surface leads to the formation of a vapor above the surface.

3) plasma formation (breakdown)



A Stark look at plasma breakdown

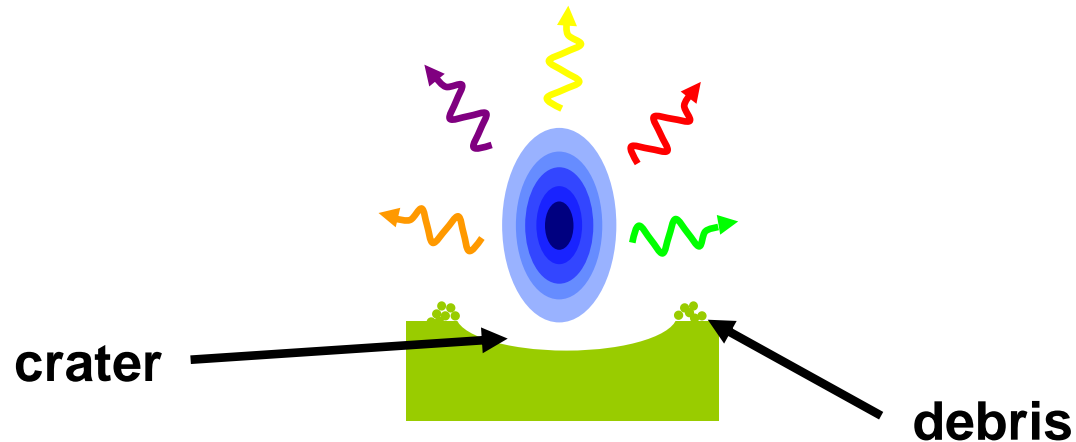


absorption of the laser
radiation by the vapor
emission
breakdown
shock wave
shock wave

- to illuminate the vapor plume.
- sub-micrometer droplets that
attering of the laser beam,
onization, and plasma formation.

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

**spontaneous emission
as atoms/ions decay to
ground state**

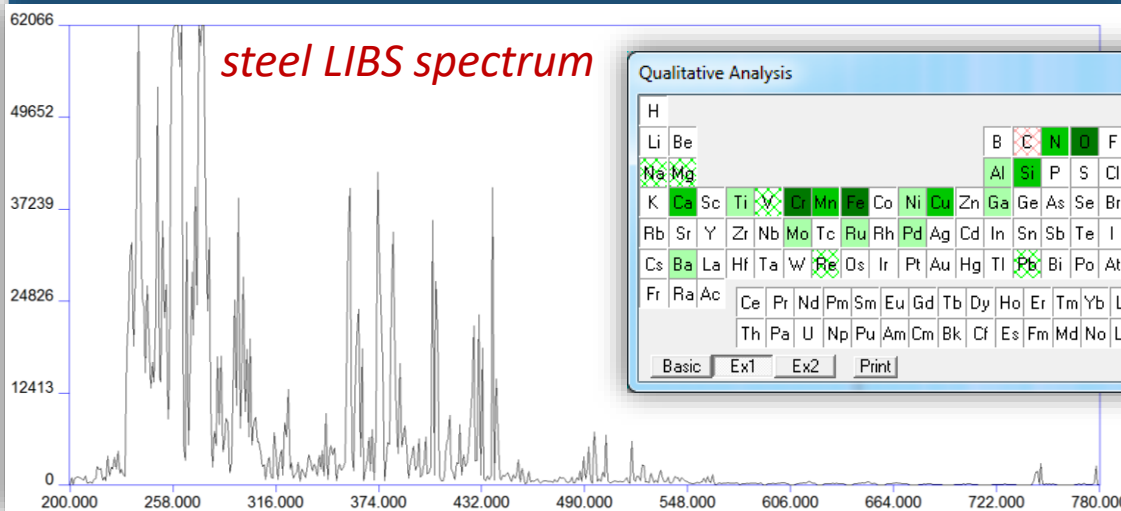


- The dynamic evolution of the plasma plume is then characterized by a fast expansion and subsequent cooling.
- Approximately 1 microsecond after the ablation pulse, spectroscopically narrow atomic/ionic emissions may be identified in the spectrum.

The Goal of LIBS Plasma Creation

- to create an optically thin plasma which is in thermodynamic equilibrium (or LTE) and whose elemental composition is proportional to the concentration of the target/sample
 - if achieved, **atomic emission spectral line intensities** can be related to **relative concentrations** of elements (sometimes absolute concentrations)

$$I_{jk} = \frac{hc}{4\pi\lambda_{jk}} A_{jk} \lambda_D \frac{N}{Z} g_k e^{-\frac{E_k}{k_B T}}$$
 - typically these conditions are only met **approximately**.

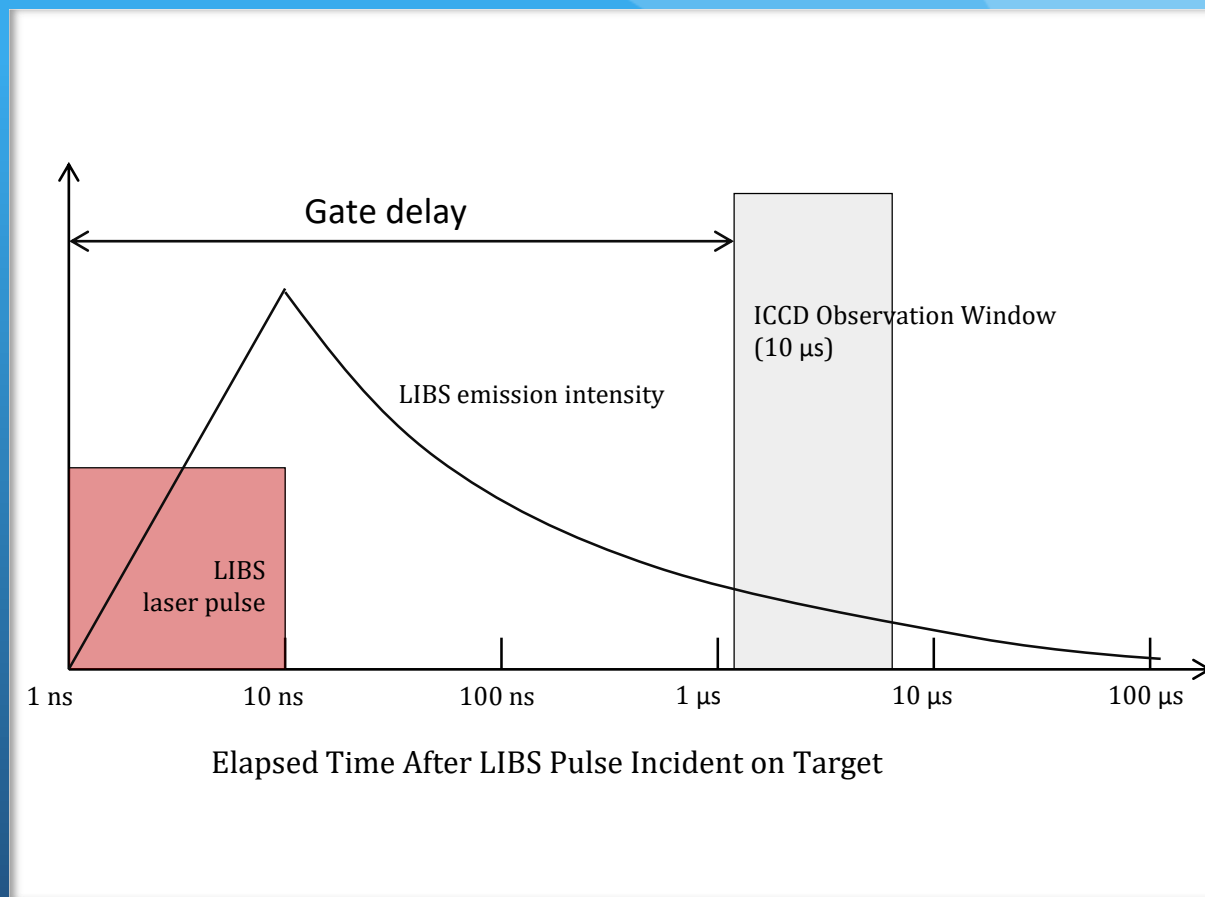


Qualitative Analysis										Element	Sensitive	Average	Week	No.	Code	Wavelength	Comment								
H									He	K	0-2	1-2	0-0	5		222.184									
Li	Be									Ca	2-2	2-3	1-3	0	hn	257.610	ok								
Na	Mg									Sc	0-0	0-0	0-0	18		259.373	? (Fe)								
K	Ca	Sc	Ti	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr	Ti	4-4	1-3	0-3	62	h	260.569	? (Fe)	
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe	V	1-3	1-4	0-3	32	v	279.827	ok
Cs	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn	Cr	3-3	3-3	2-3	0	hns	293.306	ok
Fr	Ra	Ac																Mn	4-4	3-3	1-2	0	hn	294.920	ok
			Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu		Fe	3-3	3-3	3-3	0	hns	344.200	ok
			Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lw		Co	0-2	1-4	1-3	27		470.973	nk

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

“Laser-induced breakdown spectroscopy”

or
LIBS



Outline

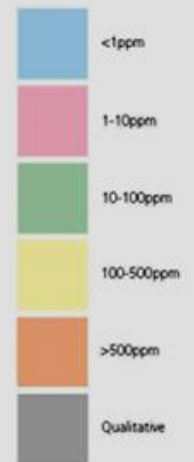
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Advantages of LIBS – sensitivity across the periodic table

Periodic table of the elements

1 H Hydrogen 1.00794																	2 He Helium 4.002						
3 Li Lithium 6.941	4 Be Beryllium 9.012182																	5 B Boron 10.811	6 C Carbon 12.0107	7 N Nitrogen 14.006424	8 O Oxygen 15.9994	9 F Fluorine 18.9984032	10 Ne Neon 20.1797
11 Na Sodium 22.989770	12 Mg Magnesium 24.3050																	13 Al Aluminum 26.981538	14 Si Silicon 28.0855	15 P Phosphorus 30.973761	16 S Sulphur 32.066	17 Cl Chlorine 35.4527	18 Ar Argon 39.948
19 K Potassium 39.0983	20 Ca Calcium 40.078	21 Sc Scandium 44.955910	22 Ti Titanium 47.867	23 V Vanadium 50.9415	24 Cr Chromium 51.9961	25 Mn Manganese 54.938049	26 Fe Iron 55.845	27 Co Cobalt 58.933200	28 Ni Nickel 58.6934	29 Cu Copper 63.546	30 Zn Zinc 65.39	31 Ga Gallium 68.723	32 Ge Germanium 72.61	33 As Arsenic 74.92160	34 Se Selenium 78.96	35 Br Bromine 79.904	36 Kr Krypton 83.80						
37 Rb Rubidium 85.4678	38 Sr Strontium 87.62	39 Y Yttrium 88.90585	40 Zr Zirconium 91.224	41 Nb Niobium 92.90638	42 Mo Molybdenum 95.94	43 Tc Technetium (98)	44 Ru Ruthenium 91.224	45 Rh Rhodium 102.90550	46 Pd Palladium 106.42	47 Ag Silver 107.8682	48 Cd Cadmium 112.411	49 In Indium 114.818	50 Sn Tin 118.710	51 Sb Antimony 121.760	52 Te Tellurium 127.60	53 I Iodine 126.90447	54 Xe Xenon 131.29						
55 Cs Cesium 132.90545	56 Ba Barium 137.327	57 La Lanthanum 138.9055	72 Hf Hafnium 178.49	73 Ta Tantalum 180.9479	74 W Tungsten 183.84	75 Re Rhenium 186.207	76 Os Osmium 190.23	77 Ir Iridium 192.217	78 Pt Platinum 195.078	79 Au Gold 196.96655	80 Hg Mercury 200.59	81 Tl Thallium 204.3833	82 Pb Lead 207.2	83 Bi Bismuth 208.98038	84 Po Polonium (209)	85 At Astatine (210)	86 Rn Radon (222)						
87 Fr Francium (223)	88 Ra Radium (226)	89 Ac Actinium (227)	104 Rf Rutherfordium (261)	105 Db Dubnium (262)	106 Sg Seaborgium (263)	107 Bh Bohrium (263)	108 Hs Hassium (265)	109 Mt Meitnerium (266)	110 (269)	111 (272)	112 (277)	113	114										
		58 Ce Cerium 140.116	59 Pr Praseodymium 140.90765	60 Nd Neodymium 144.24	61 Pm Promethium (145)	62 Sm Samarium 150.36	63 Eu Europium 151.964	64 Gd Gadolinium 157.25	65 Tb Terbium 158.92534	66 Dy Dysprosium 162.50	67 Ho Holmium 164.93032	68 Er Erbium 167.26	69 Tm Thulium 168.93421	70 Yb Ytterbium 173.04	71 Lu Lutetium 174.967								
		90 Th Thorium 232.0381	91 Pa Protactinium 231.03688	92 U Uranium 238.02891	93 Np Neptunium (237)	94 Pu Plutonium (244)	95 Am Americium (243)	96 Cm Curium (247)	97 Bk Berkelium (247)	98 Cf Californium (251)	99 Es Einsteinium (252)	100 Fm Fermium (257)	101 Md Mendelevium (258)	102 No Nobelium (259)	103 Lr Lawrencium (262)								

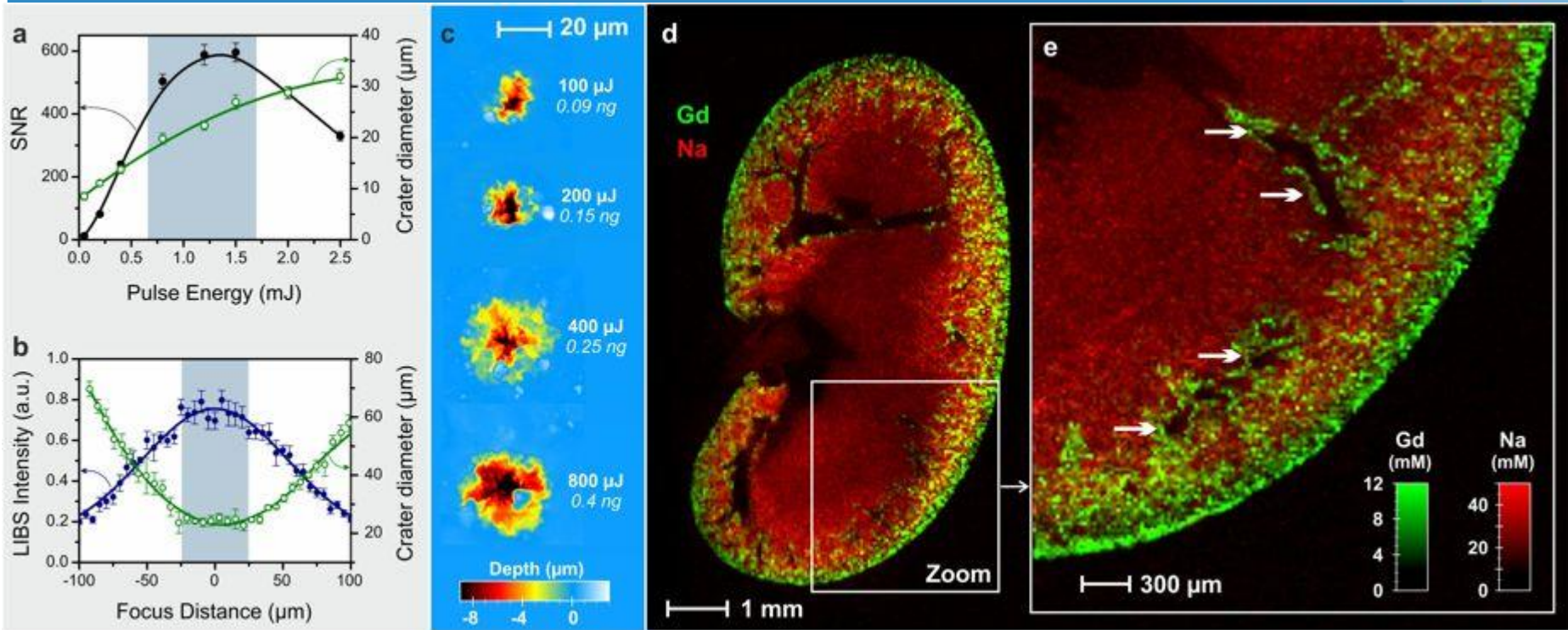
Typical detection limits for LIBS



Courtesy of Lightwind Corp.,
<http://www.lightwindcorp.com/laser-induced-breakdown-spectroscopy.html>

Advantages of LIBS - spatial resolution

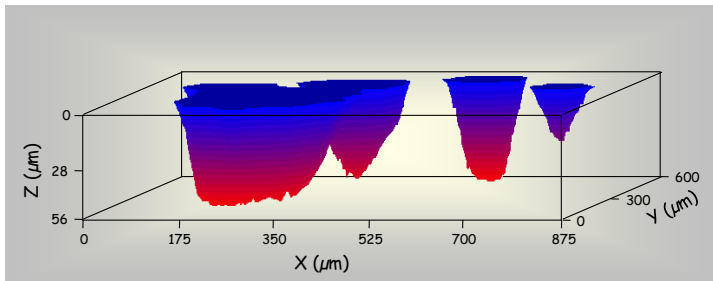
- Laser allows point sampling (1-100 micron)
- Elemental “surface maps” can then be created



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

3-DIMENSIONAL MAP OF ALUMINUM INCLUSIONS

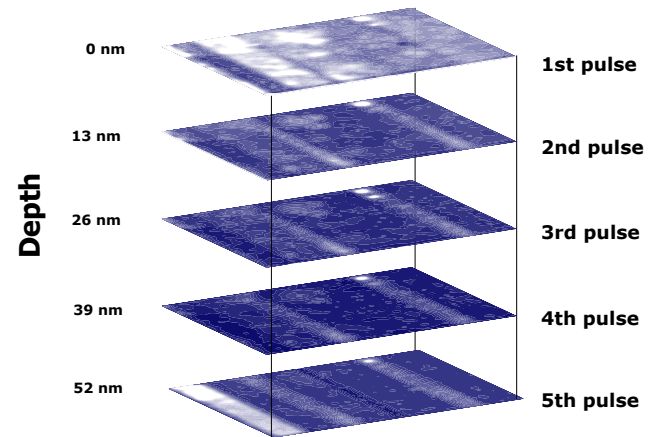


Courtesy of Ben Smith

- 13 positions
- 100 laser shots in depth
- Mapped volume = $600 \times 875 \times 56 \mu\text{m}^3$
- Repetition rate = 10 Hz
- Analysis time = 2 min and 30 s

TOMOGRAPHY

Carbon impurities on silicon wafers

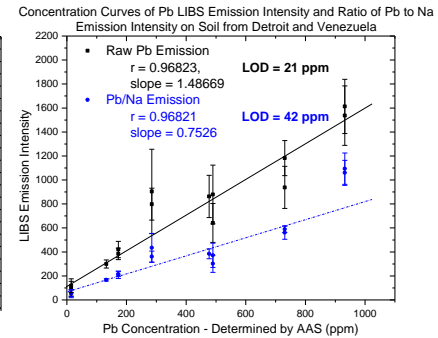
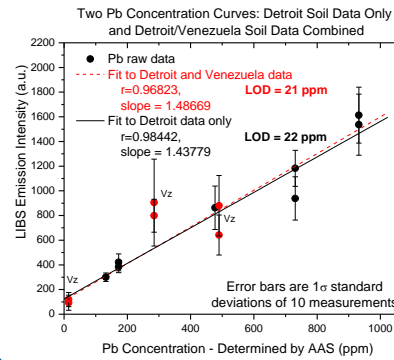


Nitrogen laser; Lateral resolution $15 \mu\text{m}$, sampling depth 13 nm

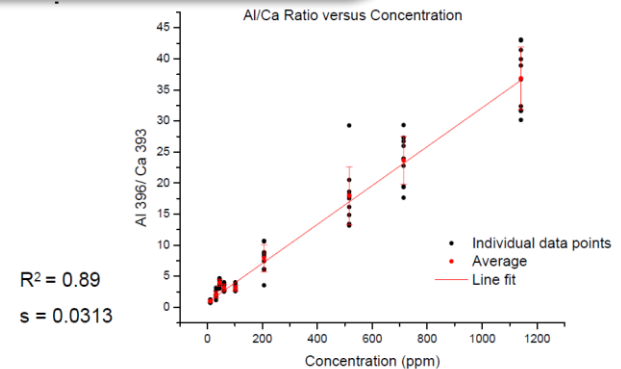
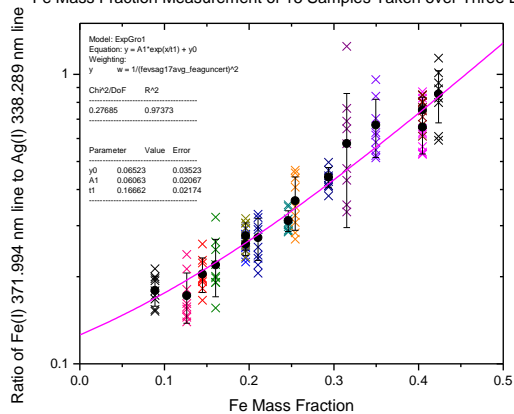
Courtesy of Ben Smith

Advantages of LIBS – sensitivity & speed

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



Fe Mass Fraction Measurement of 15 Samples Taken over Three Days



Advantages of LIBS - CBRNE prototypes have been built

Backpack contains broadband high-resolution spectrometer, laser power supply, computer, and battery



Head's-up display

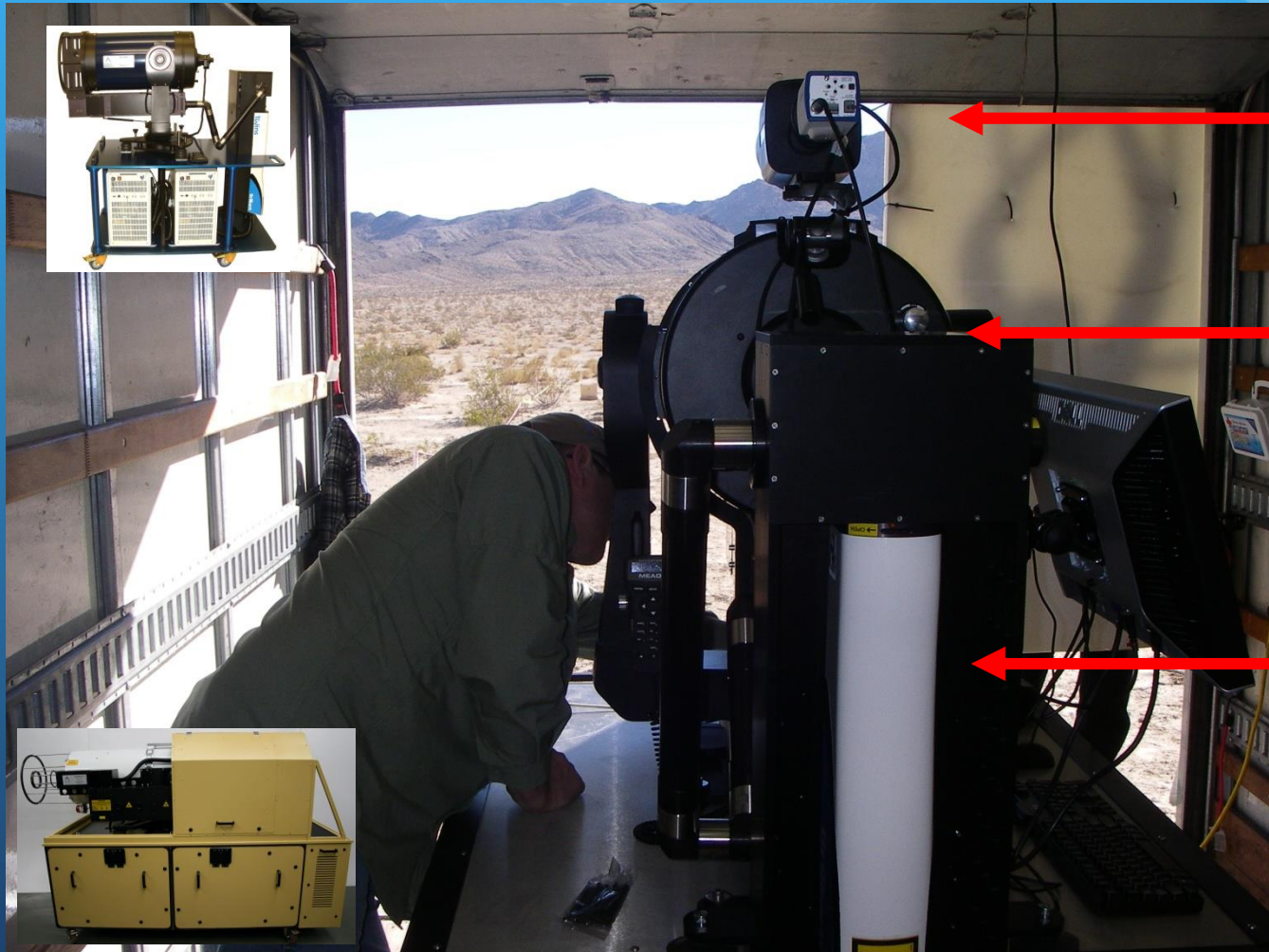
Hand-held probe contains spectrometer, joystick for control, and focus optics

Microplasma/
LIBS Event



courtesy of Ocean Optics.

Advantages of LIBS - High-energy remote systems have been built



Video Camera

Telescope

Laser Head

Advantages of LIBS - Commercial benchtop systems have been built

J200 – Applied Spectra



ChemReveal LIBS Desktop Elemental Analyzer – TSI



Advantages of LIBS – hand-held



mPulse – Oxford Instruments



LIBZ – SciApps, Inc

NanoLIBS – B&W Tek



ChemLite- TSI, Inc

EOS500 - Bruker



EOS

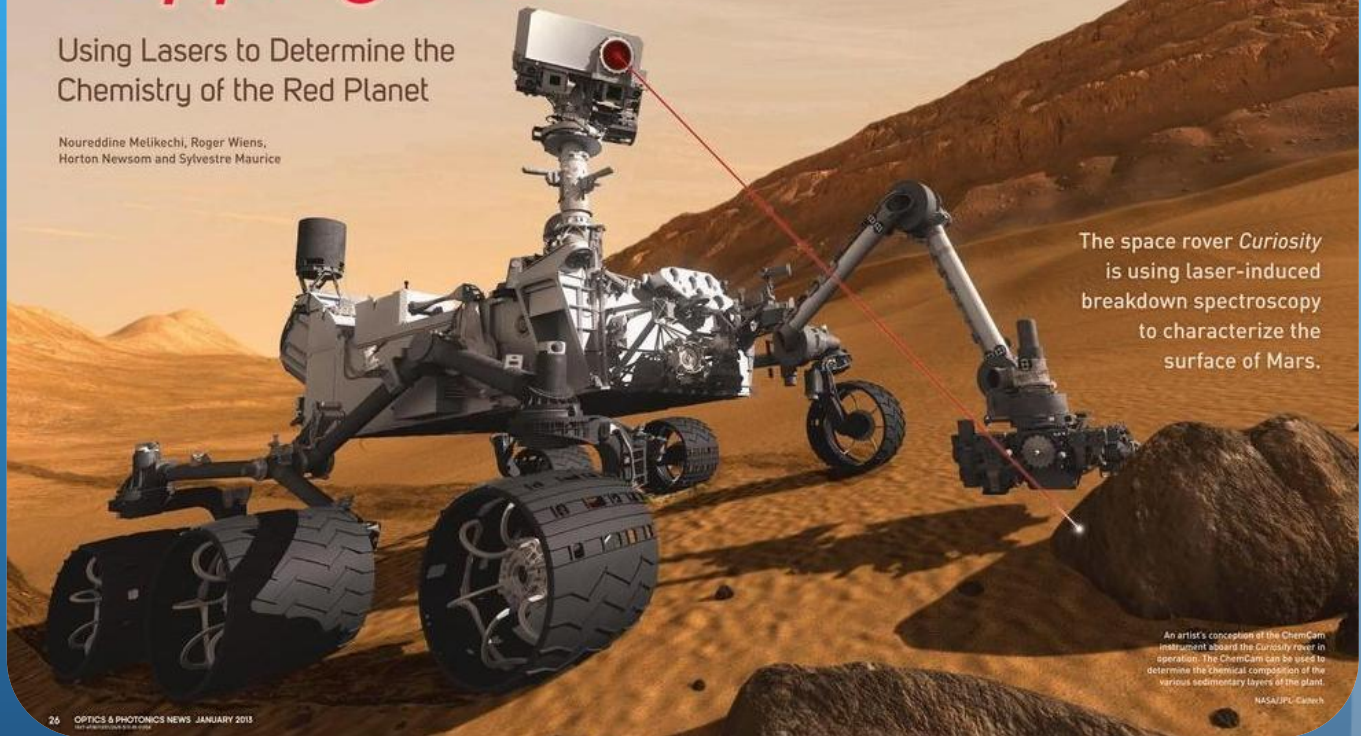
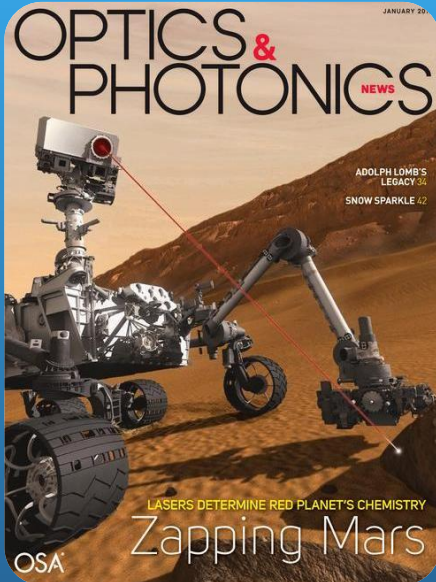
• Handheld LIBS Analyzer for Al, Ti, Mg Alloy Sorting

Advantages of LIBS – Robustness/Up-time

Zapping Mars

Using Lasers to Determine the Chemistry of the Red Planet

Noureddine Melikechi, Roger Wiens, Horton Newsom and Sylvestre Maurice

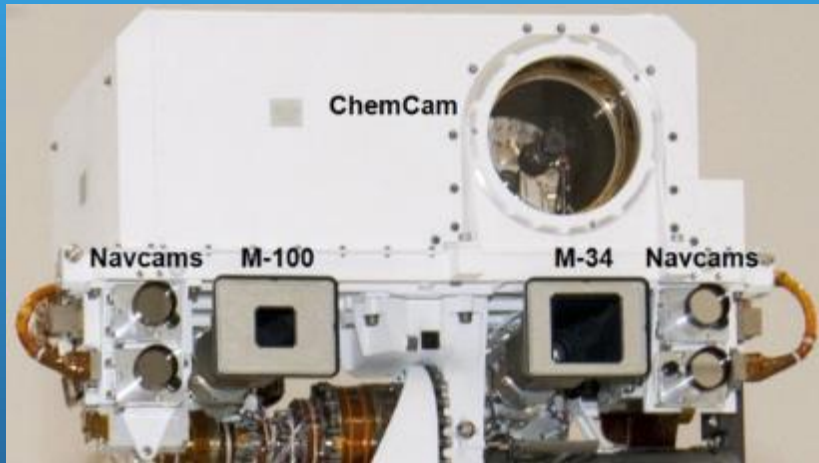


The space rover *Curiosity* is using laser-induced breakdown spectroscopy to characterize the surface of Mars.

An artist's conception of the ChemCam instrument aboard the Curiosity rover in operation. The ChemCam can be used to determine the chemical composition of the various sedimentary layers of the planet.

NASA/JPL-Caltech

Advantages of LIBS – Robustness/Up-time



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Rapid pathogen identification

Our Method of Bacteria Classification

Bacteria is cultured using trypticase soy agar (TSA).



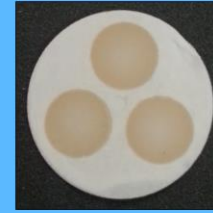
Colonies are removed and placed in 1.5 mL distilled water.



30 μ L of vortexed sample are deposited on a standard 0.22 μ m cellulose filter in contained wells.



Colloidal solution is dried forming a bacteria lawn on the clinician-friendly filter.



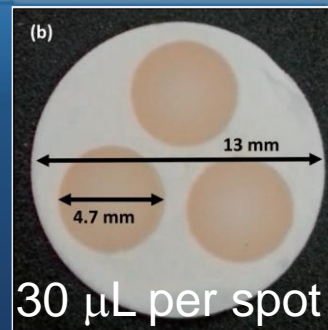
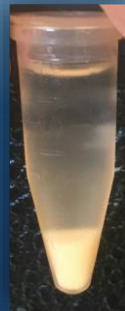
Filter is placed in an argon environment and ablated using a pulsed 1064 nm Nd: YAG laser.



Average time to complete bacterial classification = 1 hour

Average time to complete bacterial classification = 1 hour

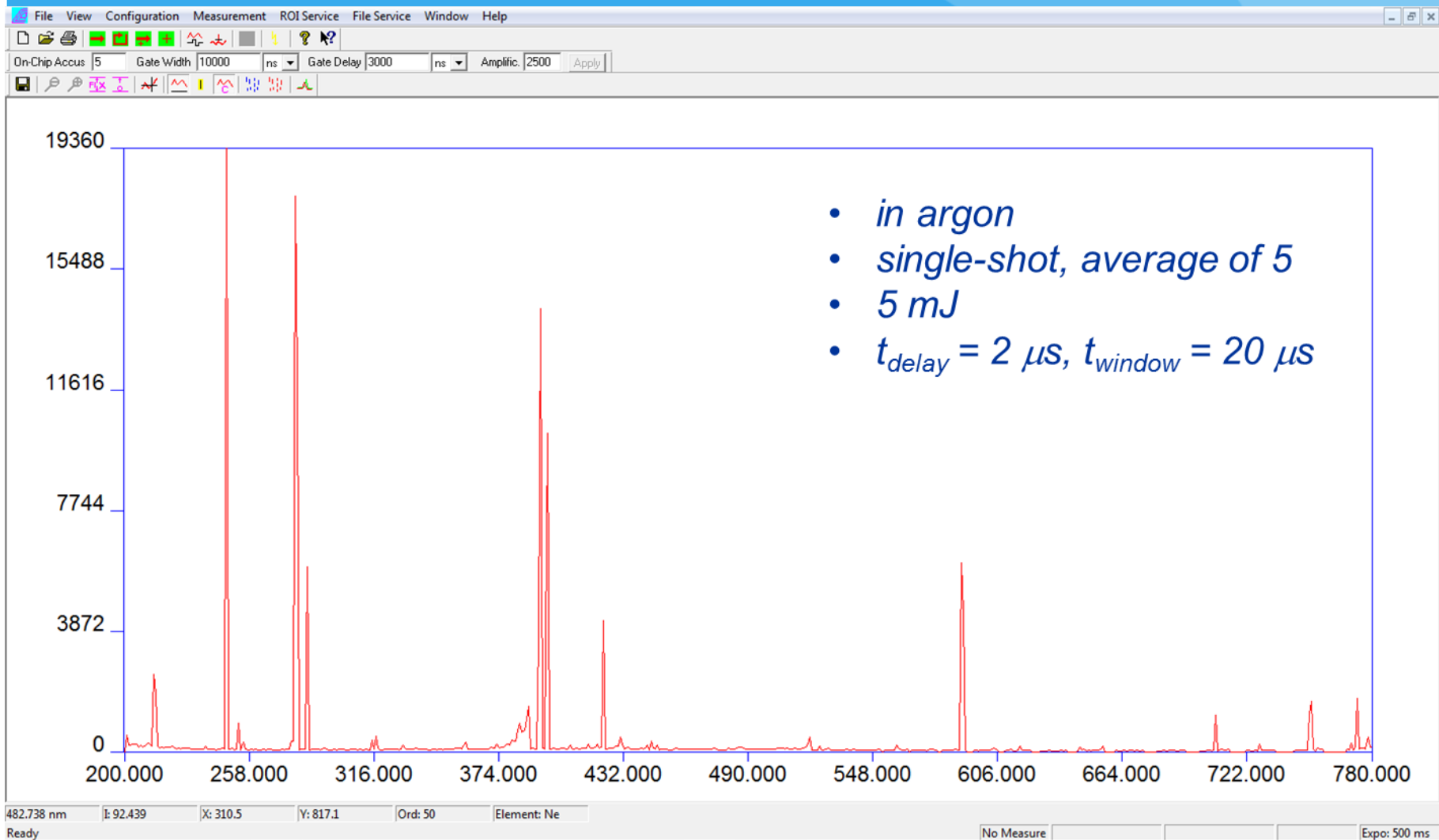
$>10^9$ cfu/ml



This is a LOT of bacteria!

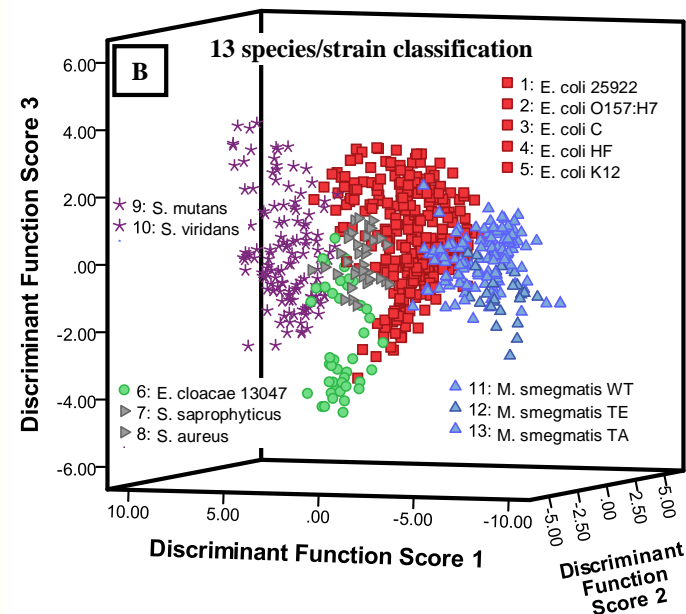
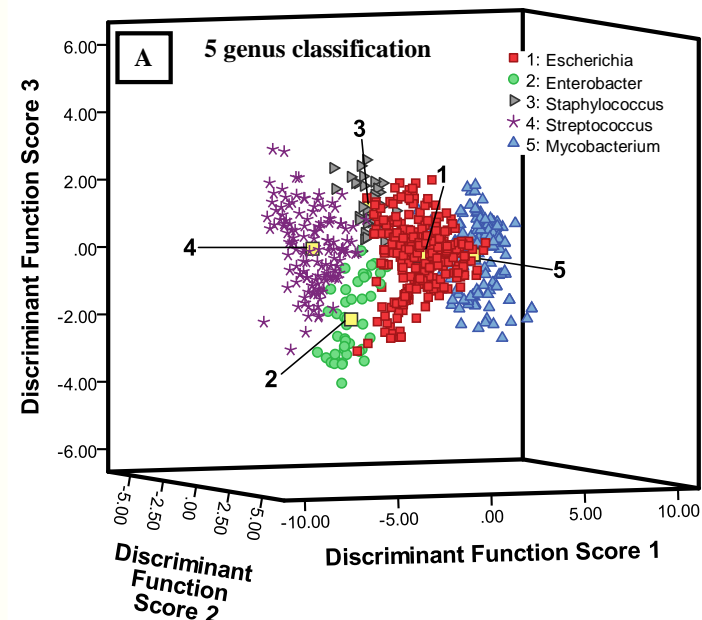
2014-2016

Variable Down Selection



How unique is “unique”?

- ✓ We can identify a bacterial species, certainly its genus, with high sensitivity and specificity (confirmed by others).
- ✓ We can differentiate strains of *E. coli* (demonstrated by others in MRSA).
- ✓ Multiple multivariate techniques effective at discriminating spectra.



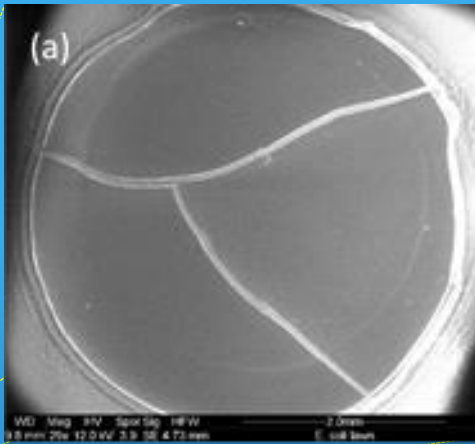
PLSDA

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

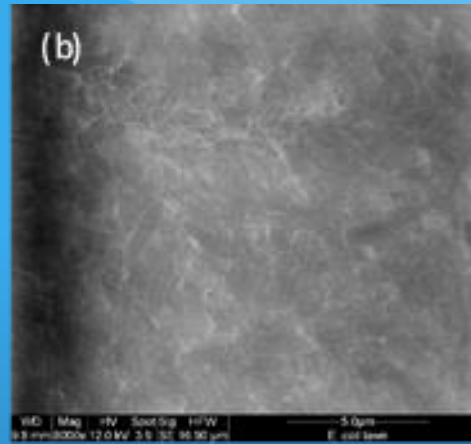
DFA

<i>E. COLI</i>	True	False
Positive	89.63%	15.95%
Negative	84.05%	10.37%
<i>STAPHYLOCOCCUS</i>	True	False
Positive	86.49%	5.85%
Negative	94.15%	13.51%
<i>STREPTOCOCCUS</i>	True	False
Positive	99.26%	13.32%
Negative	88.68%	0.74%
<i>MYCOBACTERIUM</i>	True	False
Positive	96.10%	4.08%
Negative	95.92%	3.90%

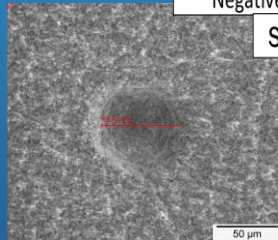
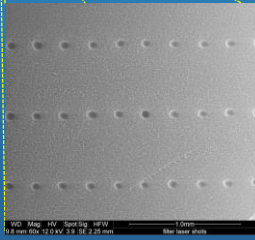
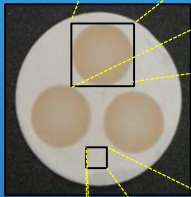
DFA: Sensitivity: $91.37 \pm 16.39 \%$ Specificity: $97.46 \pm 9.35 \%$
PLSDA: Sensitivity: $93.13 \pm 10.25 \%$ Specificity: $90.60 \pm 21.33 \%$



(a) DFA



(b) PLS-DA

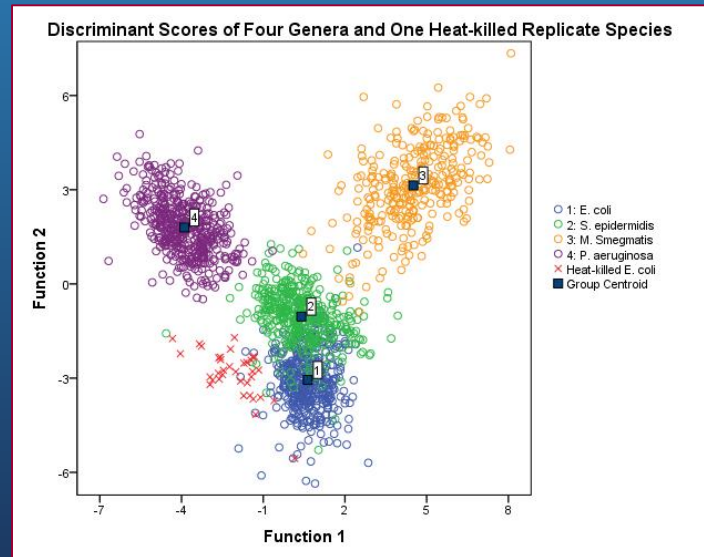


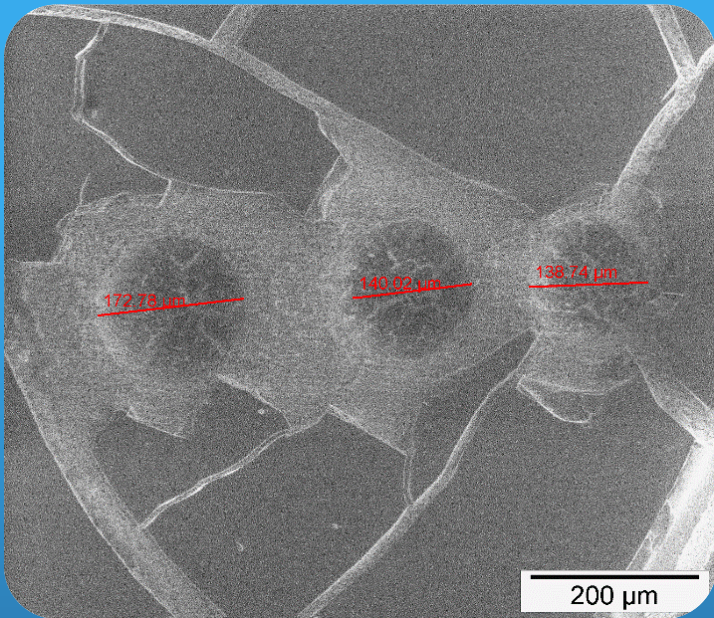
<i>Escherichia</i>	TRUE	FALSE	<i>Staphylococcus</i>	TRUE	FALSE	<i>Escherichia</i>	TRUE	FALSE	<i>Staphylococcus</i>	TRUE	FALSE
Positive	98.28%	0.77%	Positive	97.75%	1.44%	Positive	96.55%	1.12%	Positive	96.75%	1.53%
Negative	99.23%	1.72%	Negative	98.56%	2.25%	Negative	98.88%	3.45%	Negative	98.47%	3.25%
<i>Mycobacterium</i>	TRUE	FALSE	<i>Pseudomonas</i>	TRUE	FALSE	<i>Mycobacterium</i>	TRUE	FALSE	<i>Pseudomonas</i>	TRUE	FALSE
Positive	95.36%	0.33%	Positive	99.57%	0.22%	Positive	97.02%	0.41%	Positive	98.92%	0.33%
Negative	99.67%	4.64%	Negative	99.78%	0.43%	Negative	99.59%	2.98%	Negative	99.67%	1.08%

Sensitivity: 98 ± 2% Specificity: 99 ± 1% Sensitivity: 97 ± 3% Specificity: 99 ± 2%

Highly efficient discrimination still possible on nitrocellulose medium

DFA and PLS-DA perform similarly

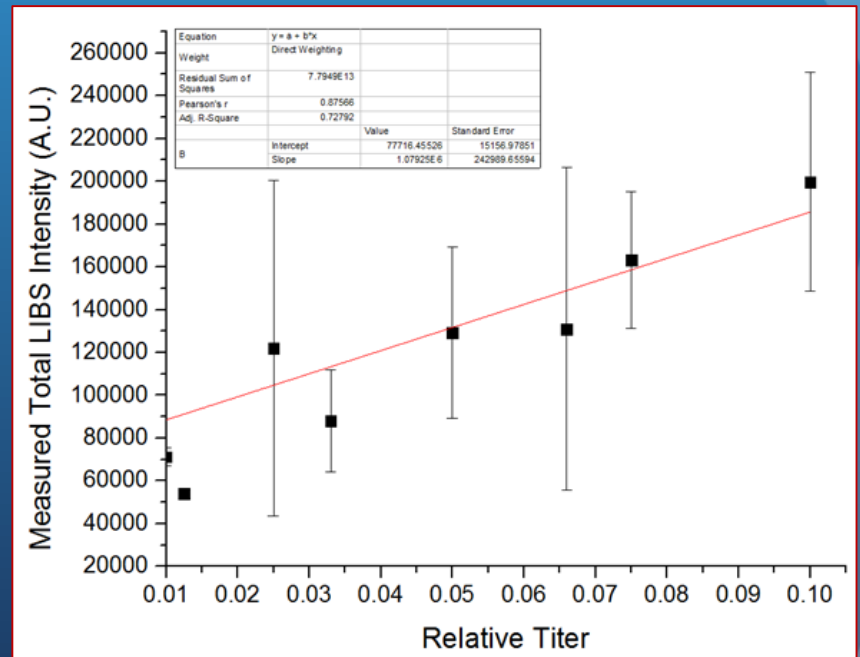
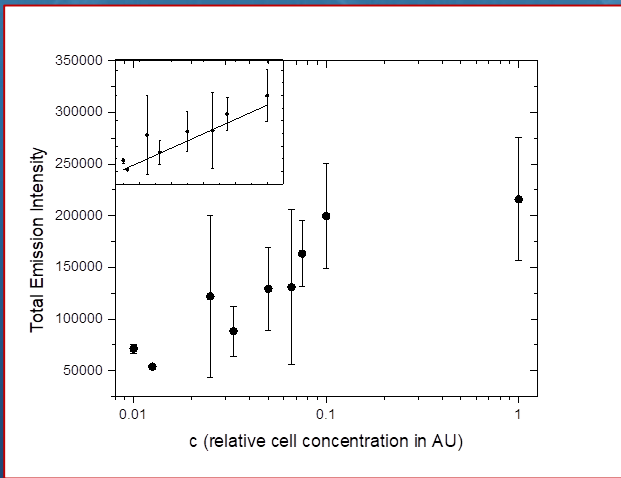




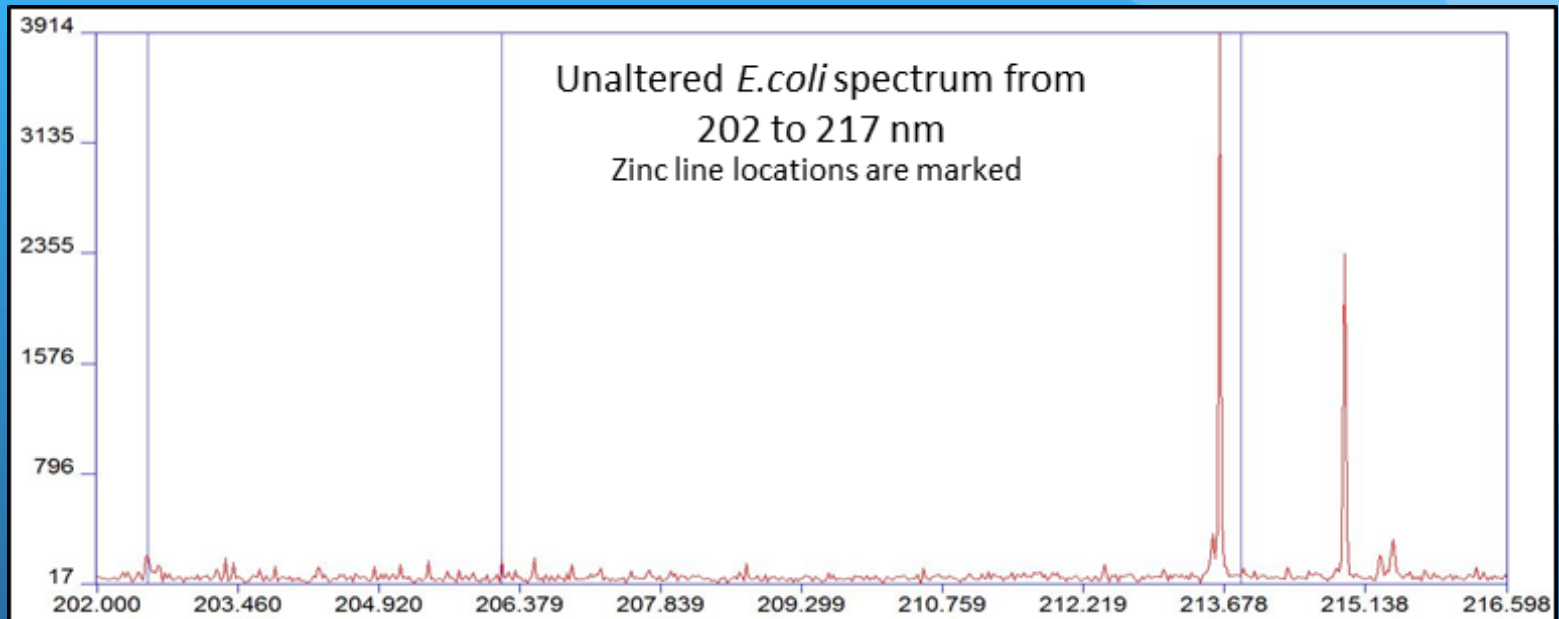
- ✓ Know ablation area
- ✓ Know bacterial titer (from absorption optical densitometry)
- ✓ Know bacterial deposition area
- **Known # cells per ablation spot**

limit of detection of 48000±12000 CFU per ablation event

500 hw

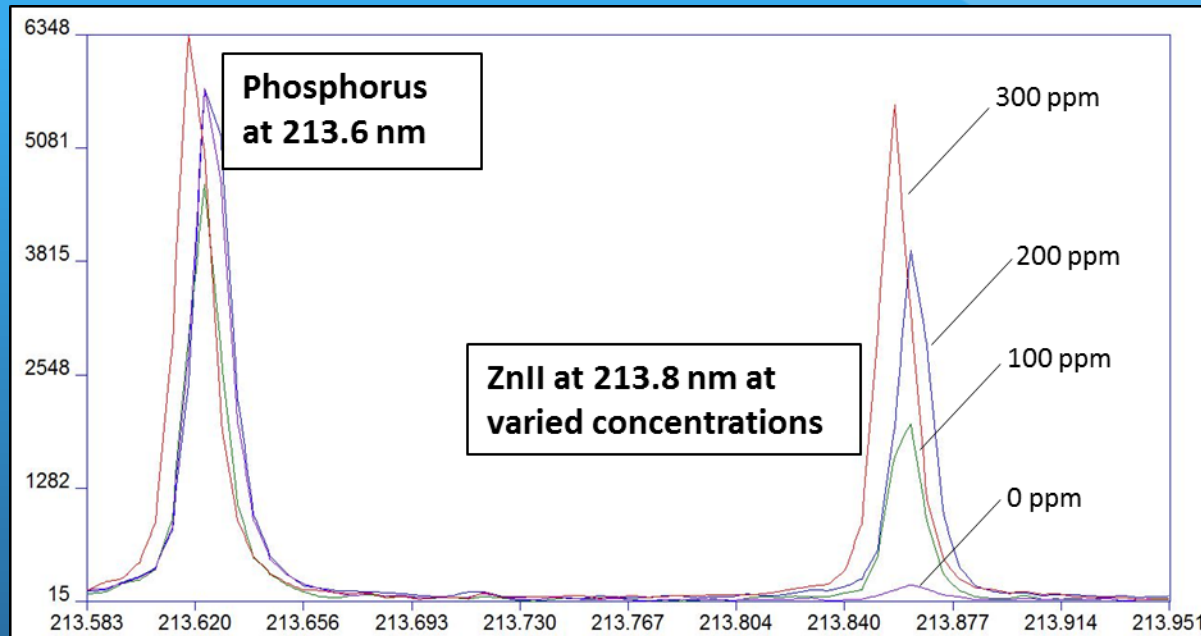


Altering Cell Metal Content: Zinc



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

Altering Cell Metal Content: Zinc



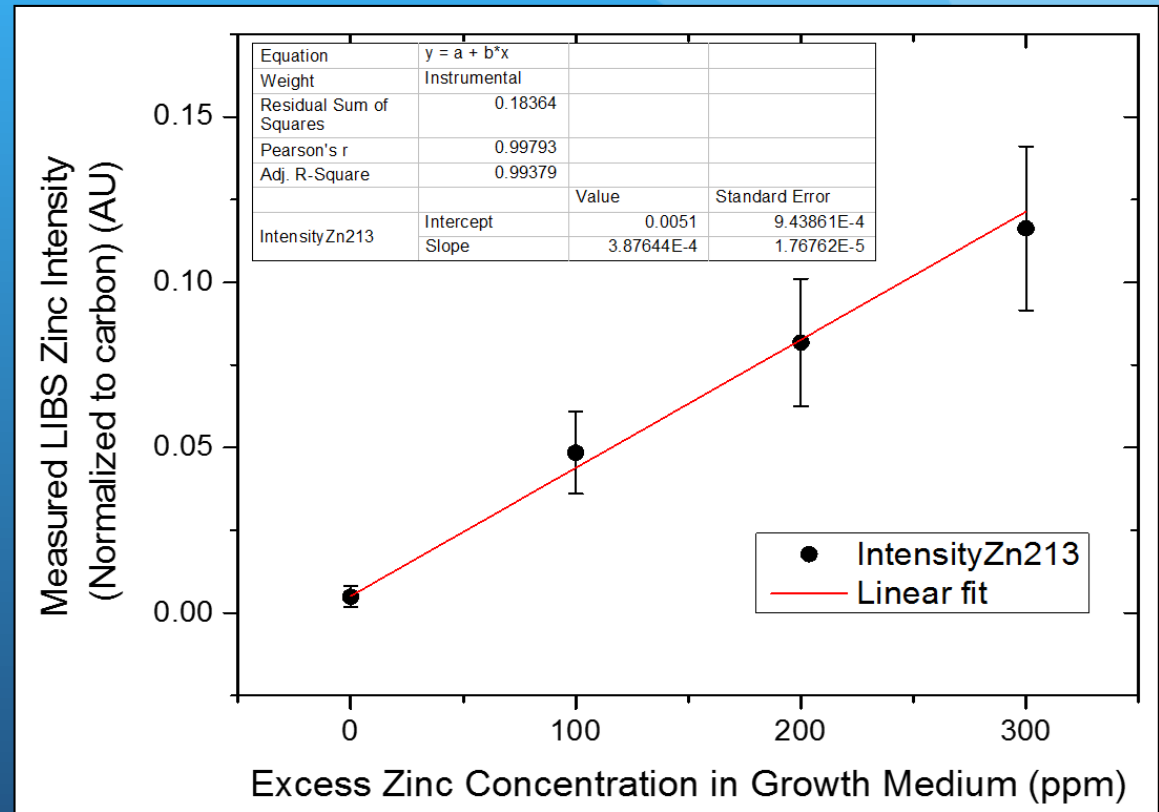
When zinc is added to the *E. coli* growth medium (TSA medium plates), cellular zinc is observed

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.

New Mounting Procedure: Concentration by Centrifugation



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

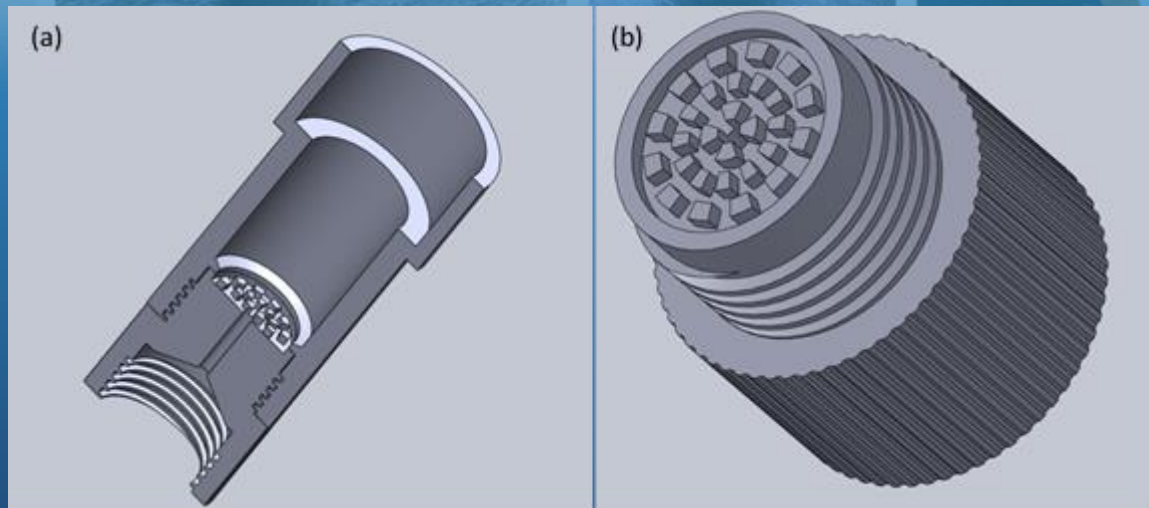
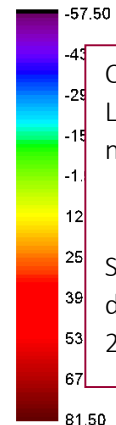
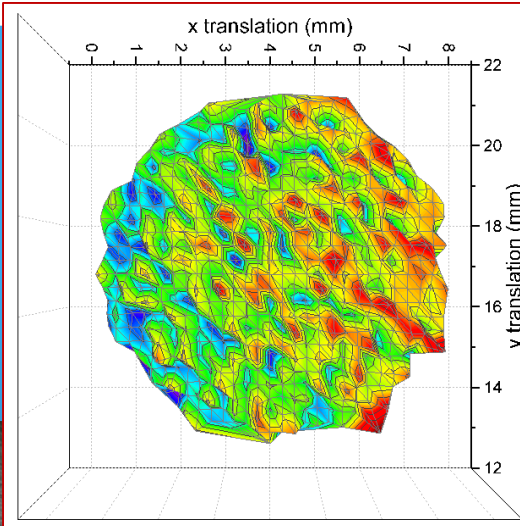


Figure 6.1: (a) Full centrifuge insert design in cross section. Filter paper is placed on the male end (b) of the device, and a seal is produced by the pressure generated by the threads. Pedestals under the filter paper prevent it from resting directly on a flat surface, allowing water to freely pass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



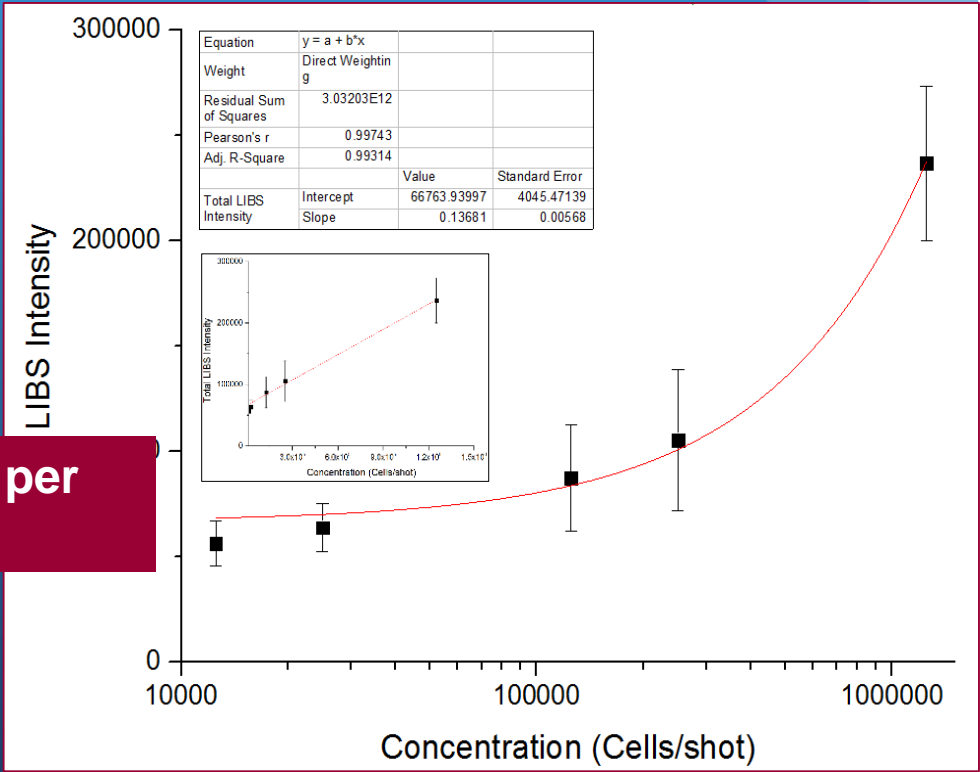
Colour map indicating percent difference of the total measured LIBS intensity from the average as a function of position on a nitrocellulose filter.

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

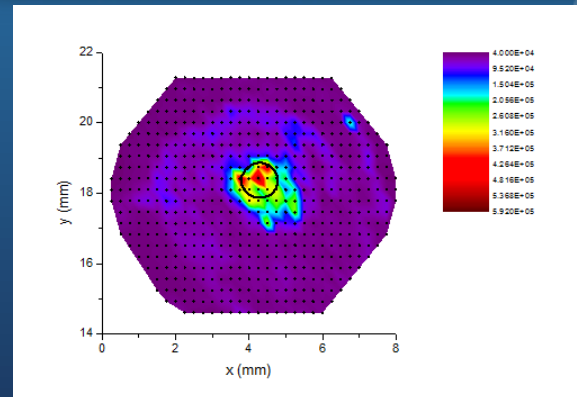


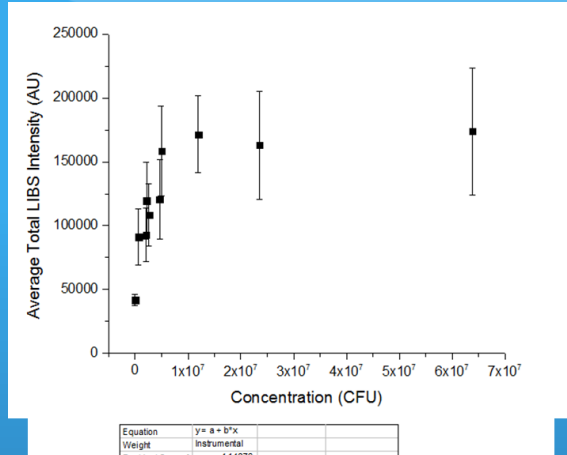
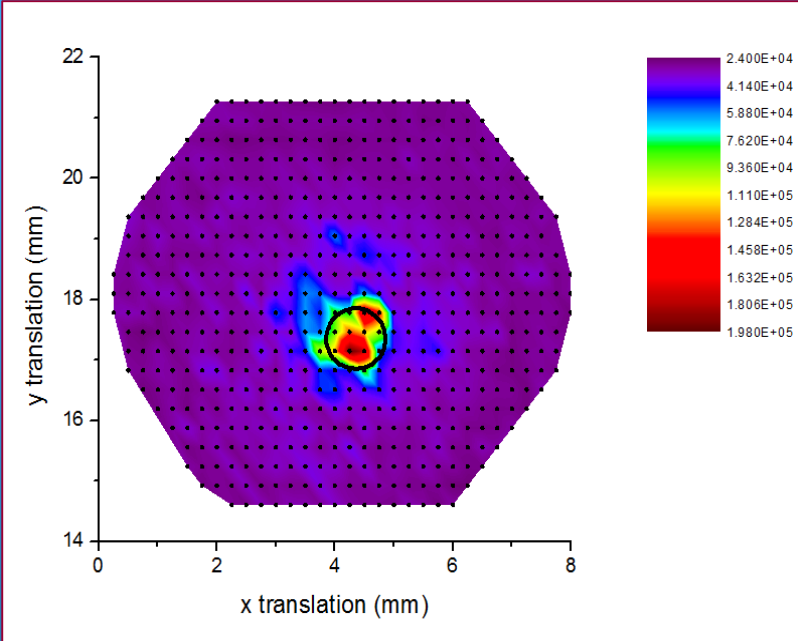
New Mounting Procedure: Concentration by Cone

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



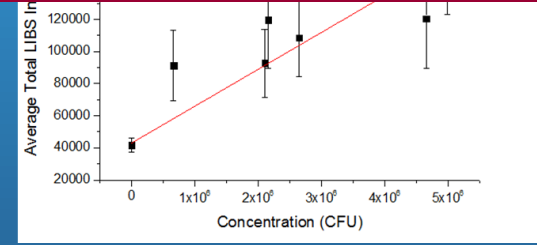
Each point on the map corresponds to a single laser shot, and the color indicates the LIBS bacterial intensity, with purple indicating no LIBS bacterial signal, and red indicating the region with the strongest LIBS bacterial signal.



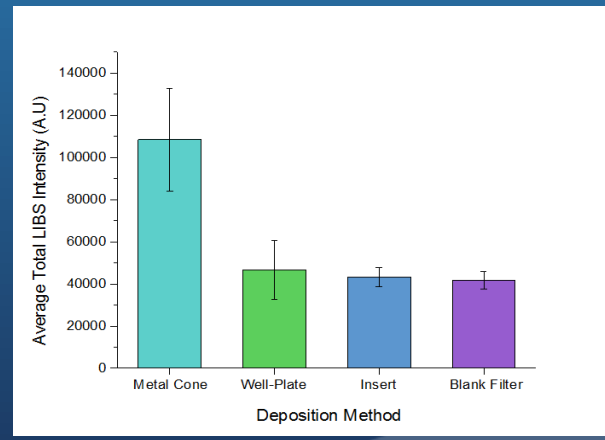
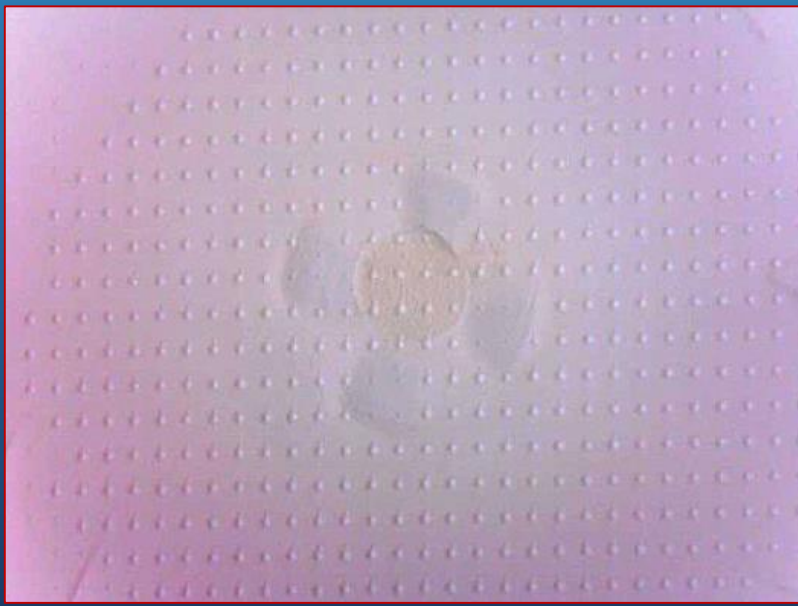


Concentration curve

limit of detection of 5530±872 CFU per ablation event



Linear region of concentration curve



Bacteria detected when other methods could not

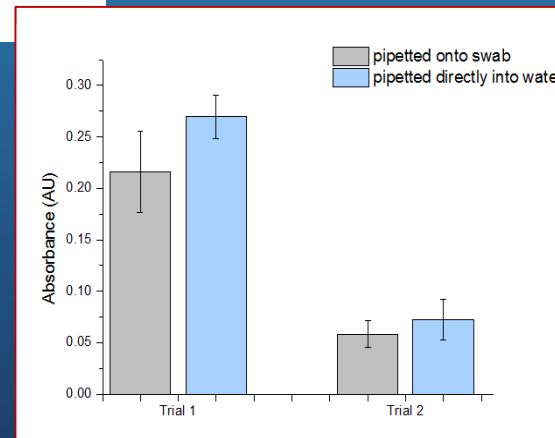
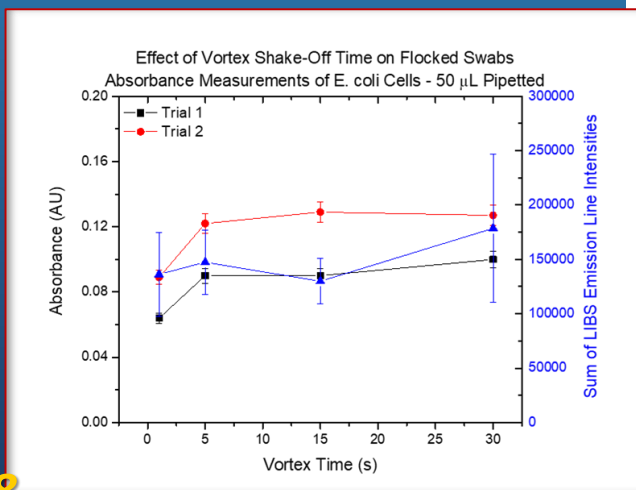
New Collection Procedure: Swabs



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

Cannot shoot right on the swab

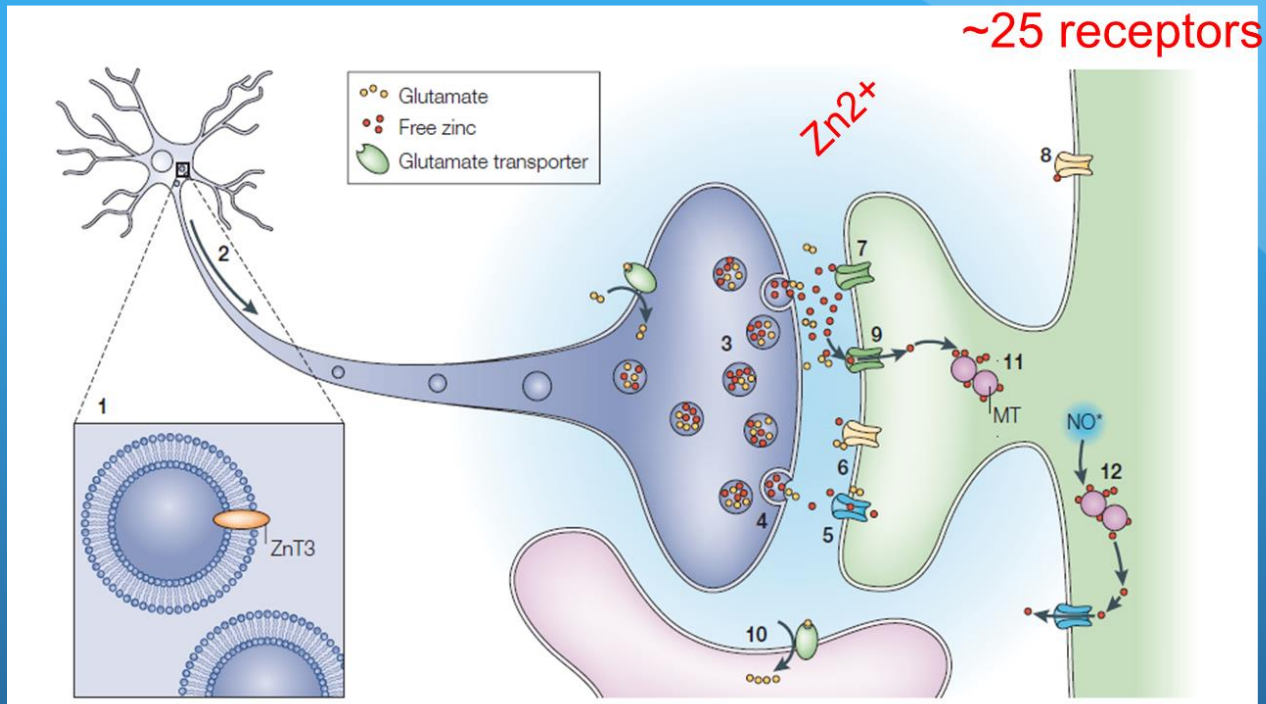
- Far too irregular (almost no plasma)
- Cells not concentrated



Outline

1. Introduction of the Method. Laser-induced breakdown spectroscopy (LIBS)
2. Comparison of LIBS with other analytic methods
3. Biomedical Applications of LIBS
 - a. A new paradigm for rapid pathogen identification
 - b. A real time assay for nutritional zinc deficiency
 - c. An ecological tool for analyzing fish otoliths
4. Concluding Thoughts

Fingernails Motivation



Frederickson et al., *Nature Neuroscience*, 2003

- Zinc deficiency is the leading cause of death among toddlers worldwide.
- It is also a leading cause of weakened immunity in the elderly.



neurobiotex, inc.



Can LIBS do this?

Why

LIBS in the brain
represent the concentration of the
body.

But we need a real time biomedical assay



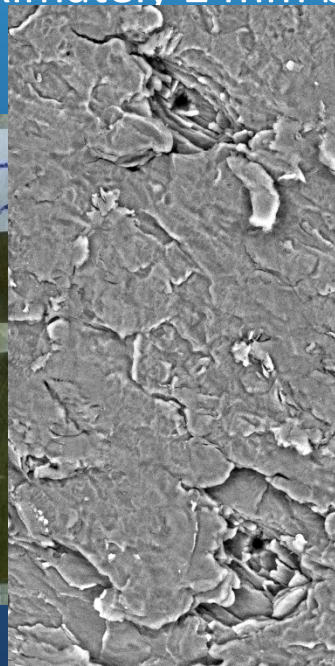
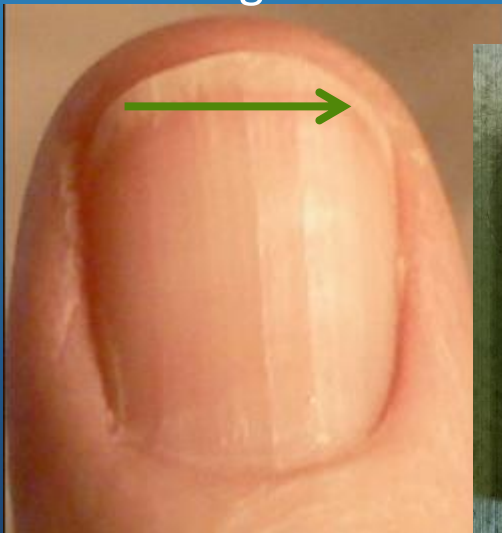
Fingernail Structure



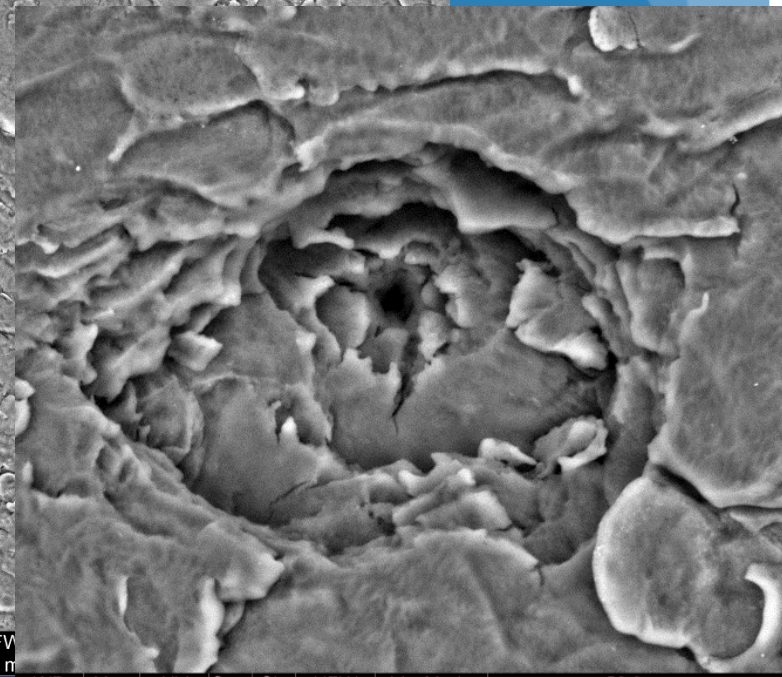
Farren, Shayler, Ennos, The Journal of Experimental Biology, 2004

Preparation of Nails

- Nail clippings of the index, middle and ring fingers (both right and left hands) of 5 subjects were taken → a total of 6 nail clippings per subject.
- Clippings were cleaned with acetone in an ultrasound bath for 10 minutes and allowed to dry for 20-30 minutes.
- Clippings are cut into approximately 2 mm by 1 mm fragments to provide a flat target.

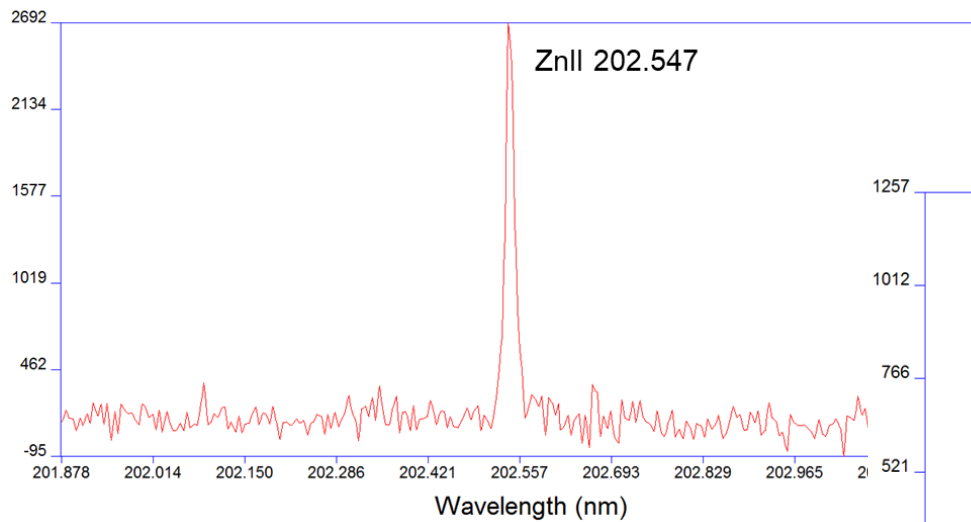


WD 10.2 mm Mag 250x HV 18.0 kV Spot 5.4 Sig BSE HFW 0.54 m

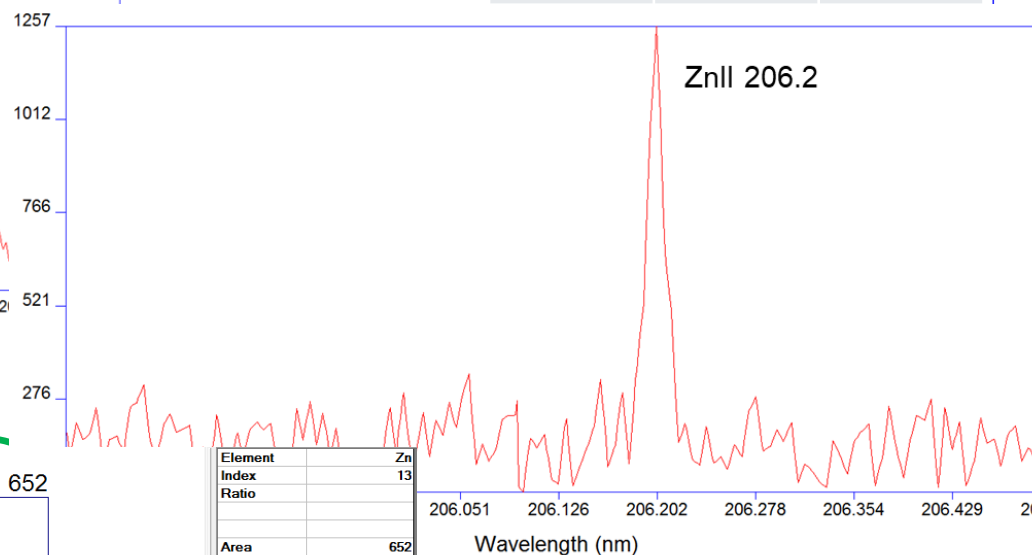


WD 9.6 mm Mag 1000x HV 18.0 kV Spot 3.9 Sig BSE HFW 0.14 mm VacMode Low vacuum S7 High 50.0 μm

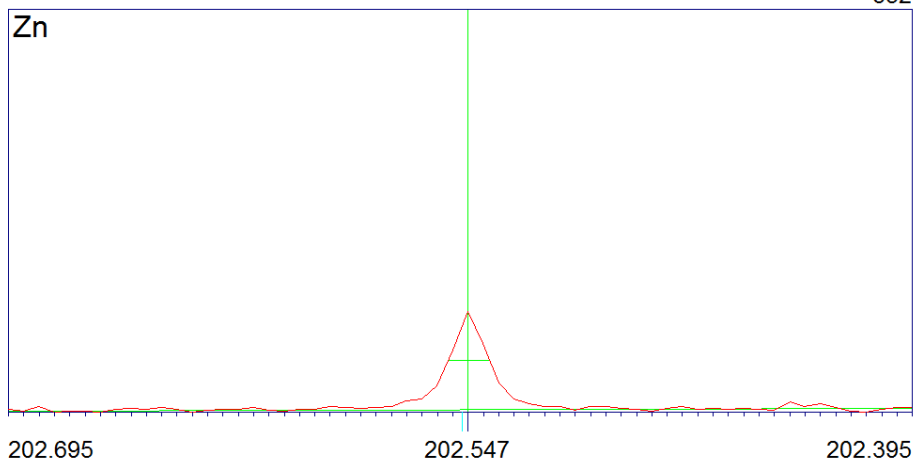
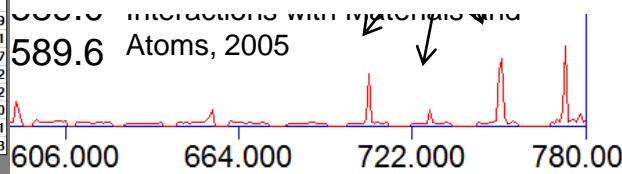
Zinc easily visible



Element	Mean (ppm)	±Std.Dev.
Mg	570.8	±511.5
Al	837.4	±427.2

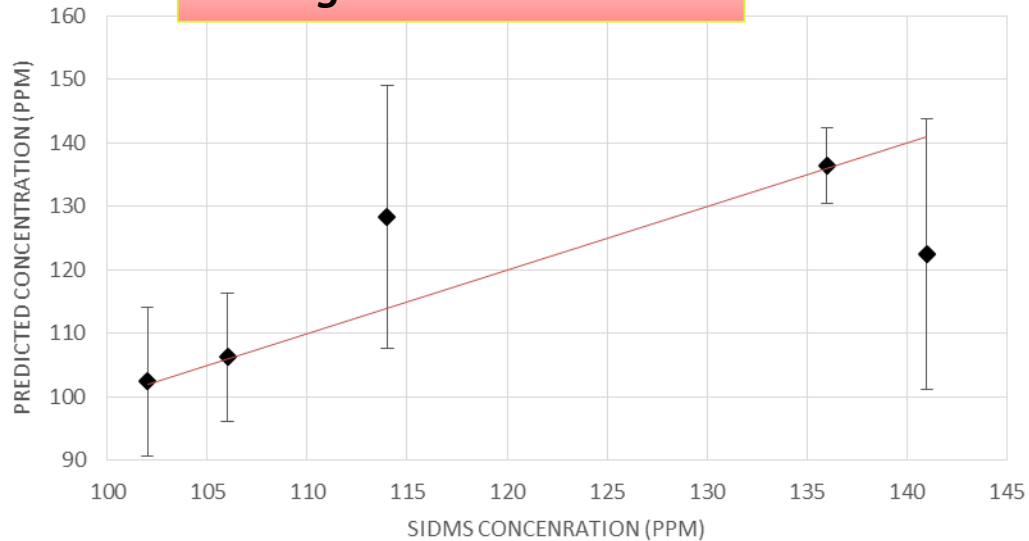


Element	Zn
Index	13
Ratio	
Area	652
Maximum	3719
Minimum	3381
Offset	3387
Height	332
Width Half M	2.72
Position	30.0
SC Fac	1
Stdev	6.43



Results

PLS regression model



- 10 laser pulses per location
- 5 locations averaged per spectrum. (i.e. 50 laser shots per spectrum).
- 30 spectra per data point (i.e. 1500 laser shots, with 1 σ st. dev. shown)

a PLS regression model was built from Zn measurements on the left hand of five volunteers and used to test the Zn measurements on their right hand.

Yielded predictions that differed from the actual concentration by an average of 6.8 ppm and a standard deviation of 14 ppm, or 12% fractional uncertainty.

4 Things to know about otoliths

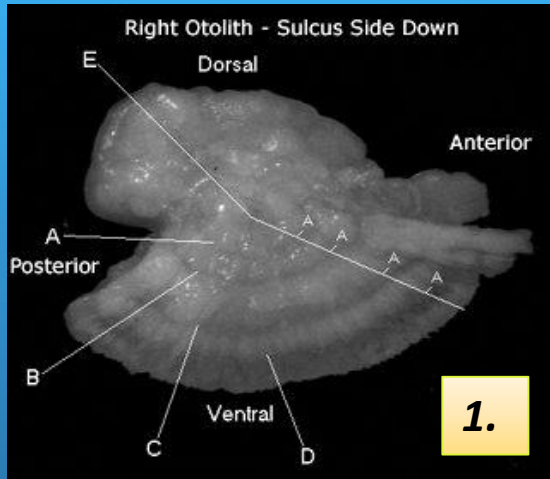


Image acquired from <http://wgosm.npafc.org/MarkFAQ.asp>

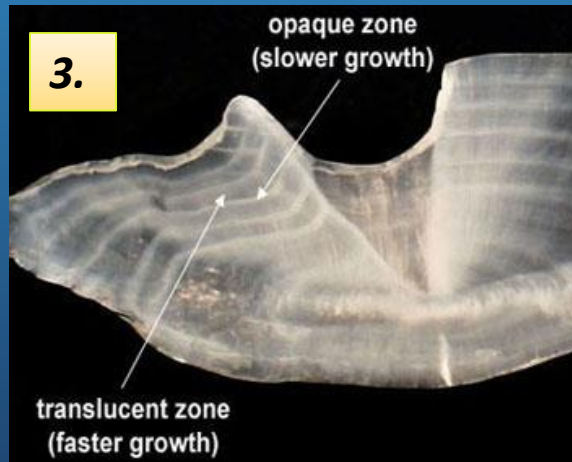
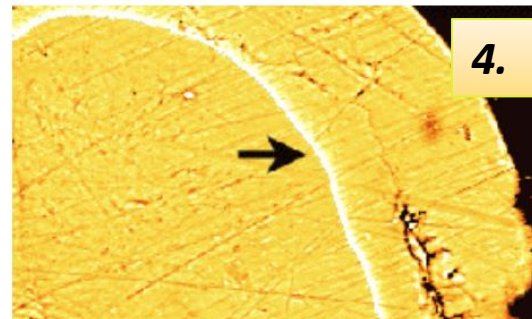


Image acquired from <http://http://keywordsuggest.org>



Photo by Ned Rozell, courtesy of <http://www.sitnews.us/>

Strontium

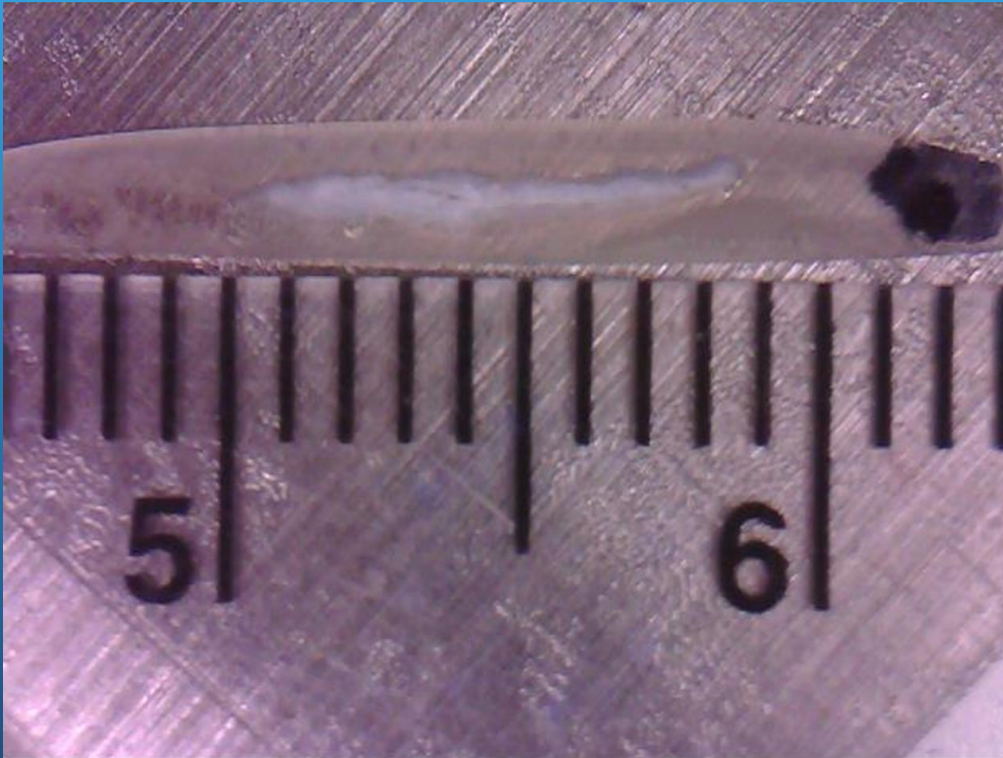


Salmonids can be successfully mass-marked using strontium chloride at any life history stage. Thermal marks, in contrast, can only be applied during a 2 to 4 week period after the eyes form in the embryos. One drawback of the strontium mark, however, is that they cannot be viewed using a traditional microscope. They are only detectable using an electron microscope equipped with an electron backscatter detector.

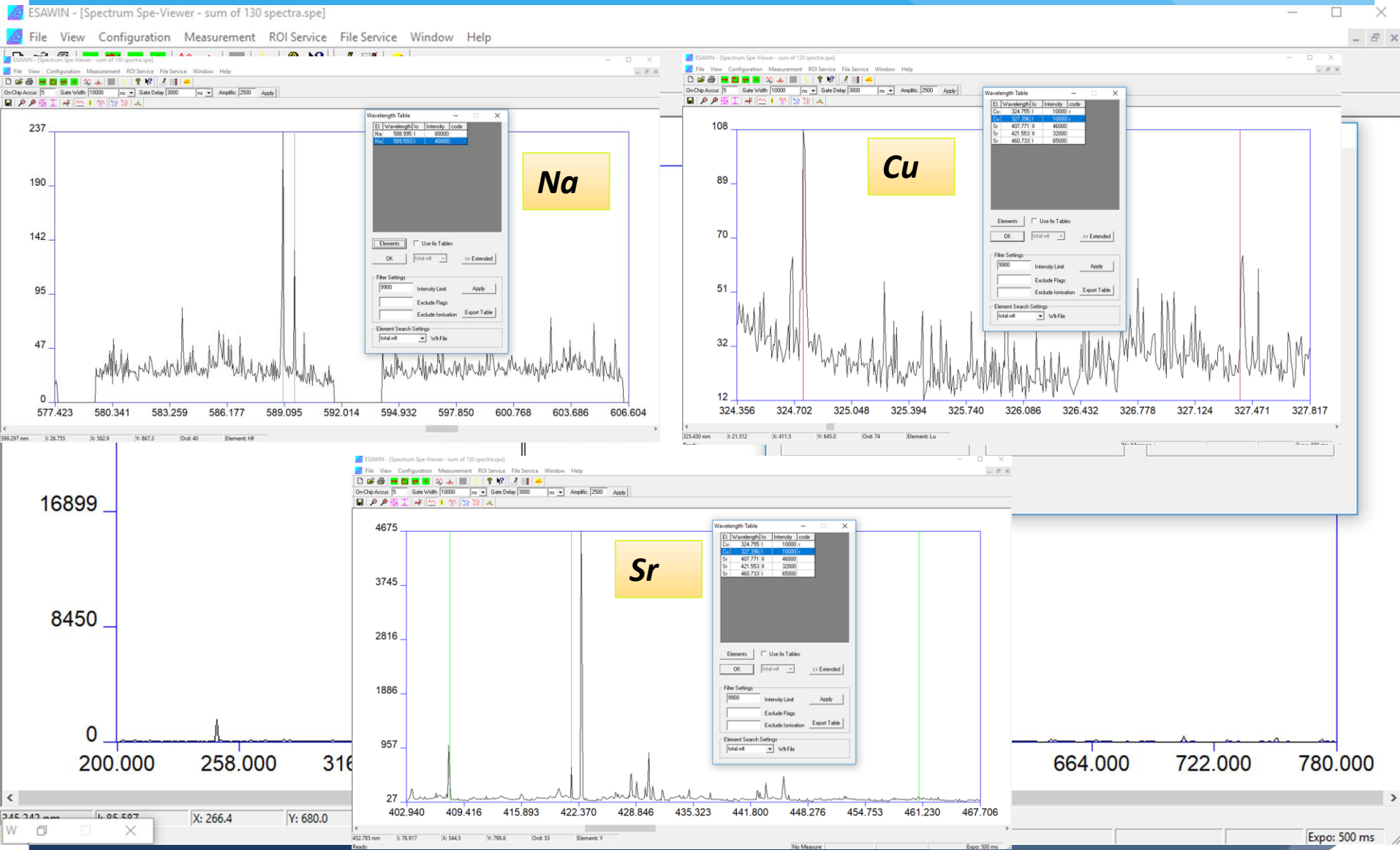
Can we monitor fresh/salt water migration via the elemental concentration?

Otolith samples prior to LIBS ablation

Mounted in a paraffin wax and cross-sectioned



LIBS Otolith Spectra



Funding and Acknowledgements

We gratefully acknowledge funding for this project provided by:

- A [Natural Sciences and Engineering Research Council of Canada](#) Discovery grant and a Research Tools and Instruments grant



- A [Canada Foundation for Innovation](#) Leaders Opportunity Fund grant

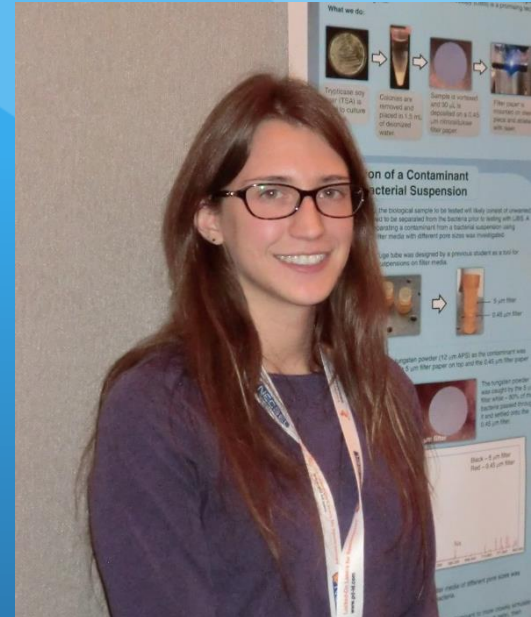


- An [Ontario Research Fund](#) Small Infrastructure Funds grant
- [University of Windsor](#) Outstanding Scholars program
- [University of Windsor](#) Faculty of Science



All Credit to the Students!

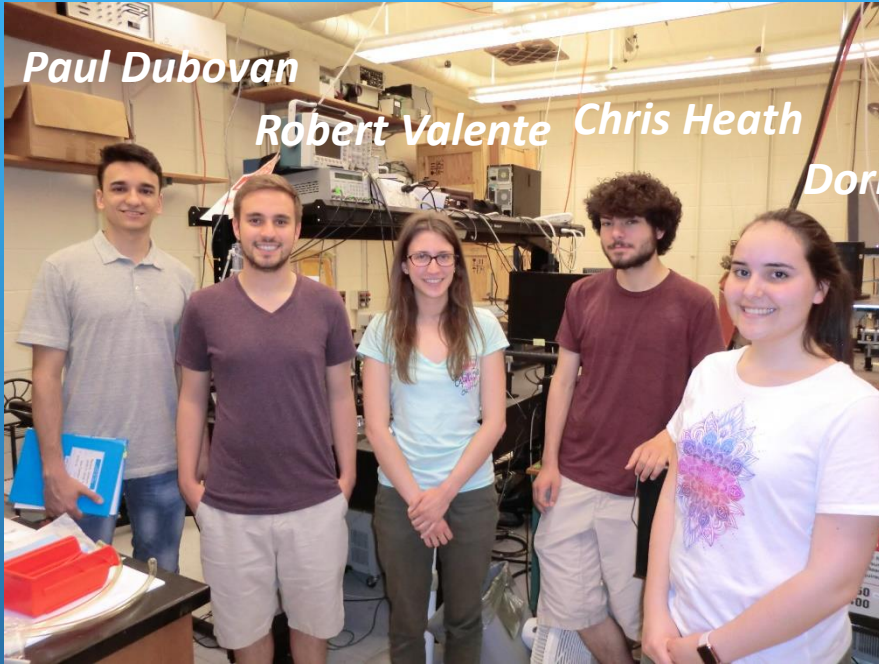
Allie Paulick



Paul Dubovan

Robert Valente Chris Heath

Doris Rusu



Dylan Malenfant



Kevin Beaugrand

Mark Armstrong