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)

**Steven Rehse, Alexandra Paulick,
Christopher Heath, Robert Valente, Paul Dubovan, Kevin Beaugrand, Mark Armstrong, Doris Rusu**

Department of Physics, University of Windsor

Staph. epidermidis

University of Windsor

Windsor, Ontario, Canada

Outline

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

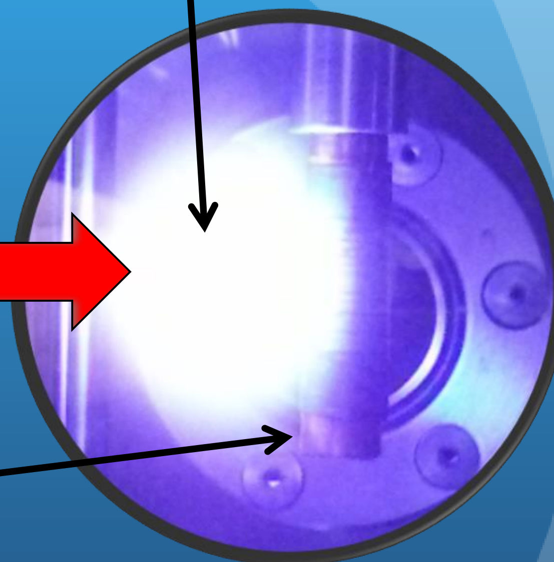
2. Comparison of LIBS with other analytic methods

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

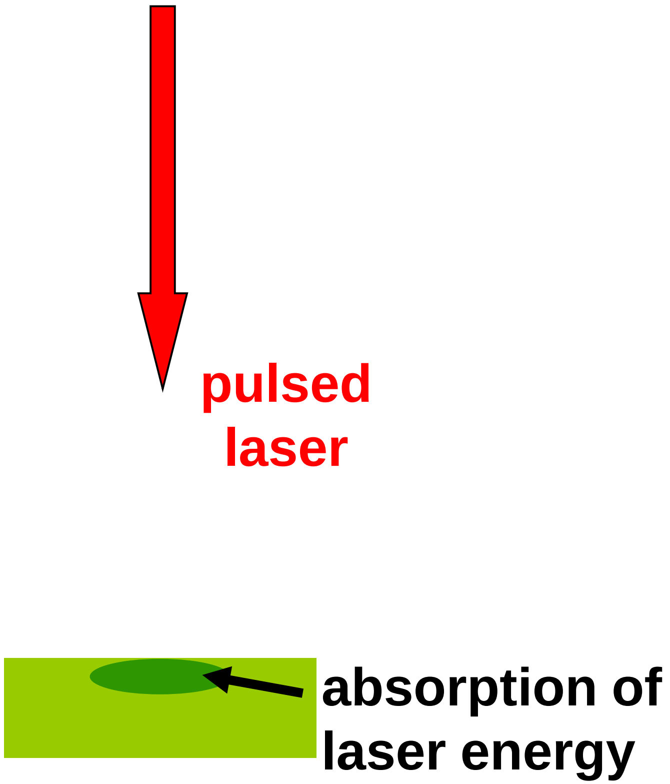
Laser-Induced Plasmas

- What is a “laser-induced plasma?”



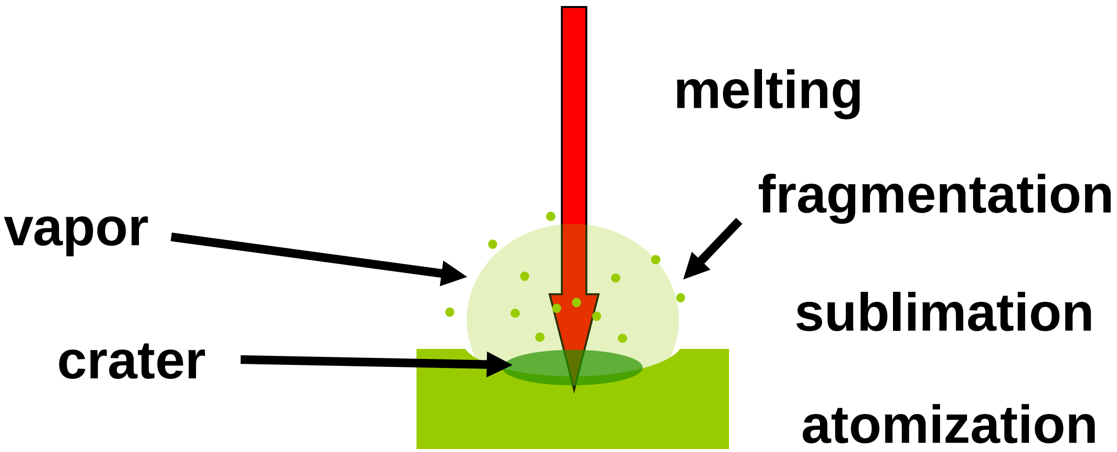
- Can be done with ns, ps, or fs lasers
- Threshold irradiance: $\sim 10^{10} - 10^{11}$ W/cm
(typically 1-100 mJ/pulse)

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.

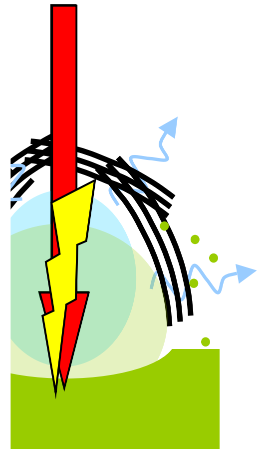
2) removal of samples mass (ablation)



- 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)



absorption of the laser radiation by the vapor

continuum emission

electrical breakdown and plasma formation

shock wave

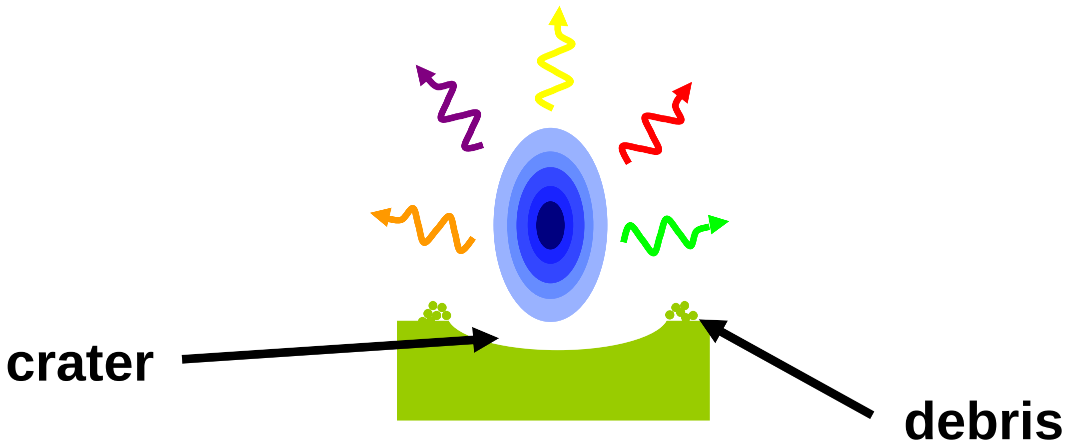
bremsstrahlung

s to illuminate the vapor plume.

o 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



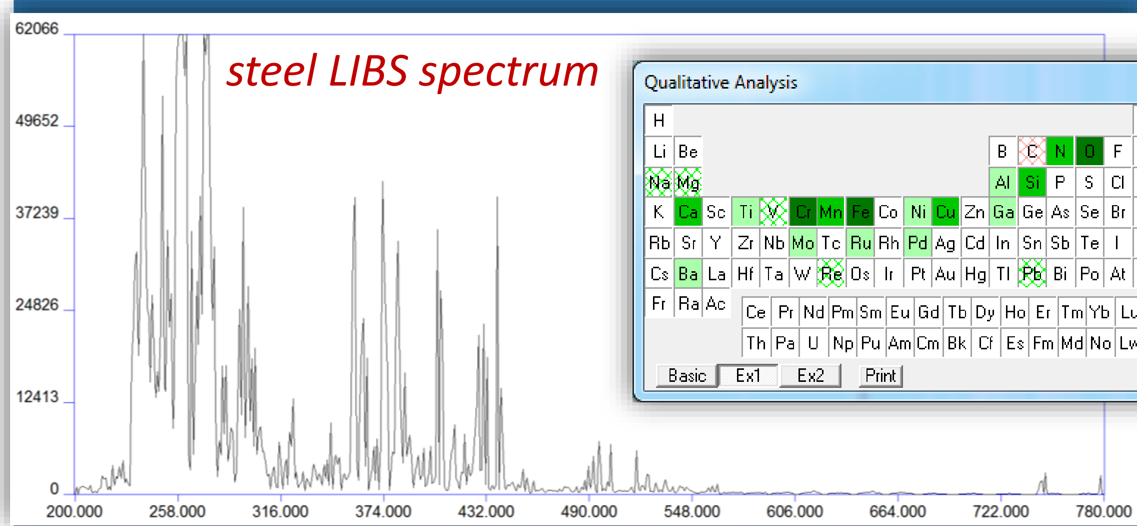
- The dynamic evolution of the plasma plume is then characterized by a fast expansion and subsequent cooling.
- Approximately 1 microsecond after the ablation pulse, spectroscopically narrow atomic/ionic emissions may be identified in the spectrum.

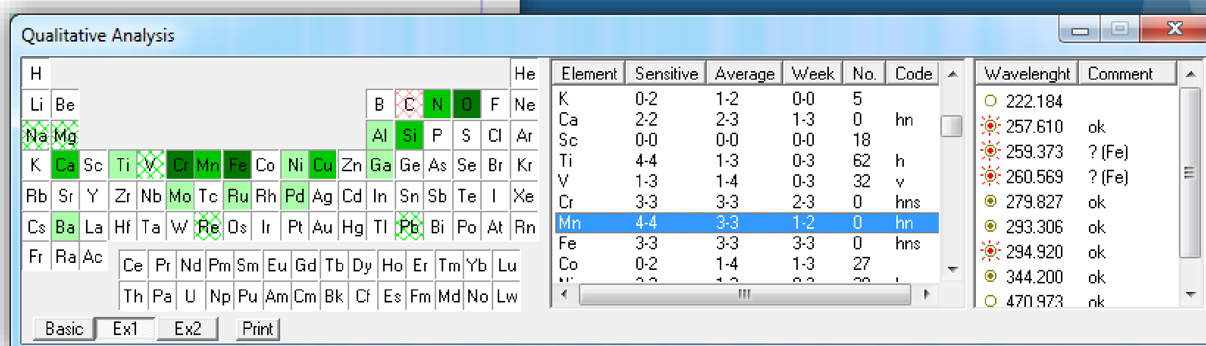
The Goal of LIBS Plasma Creation

- 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***.



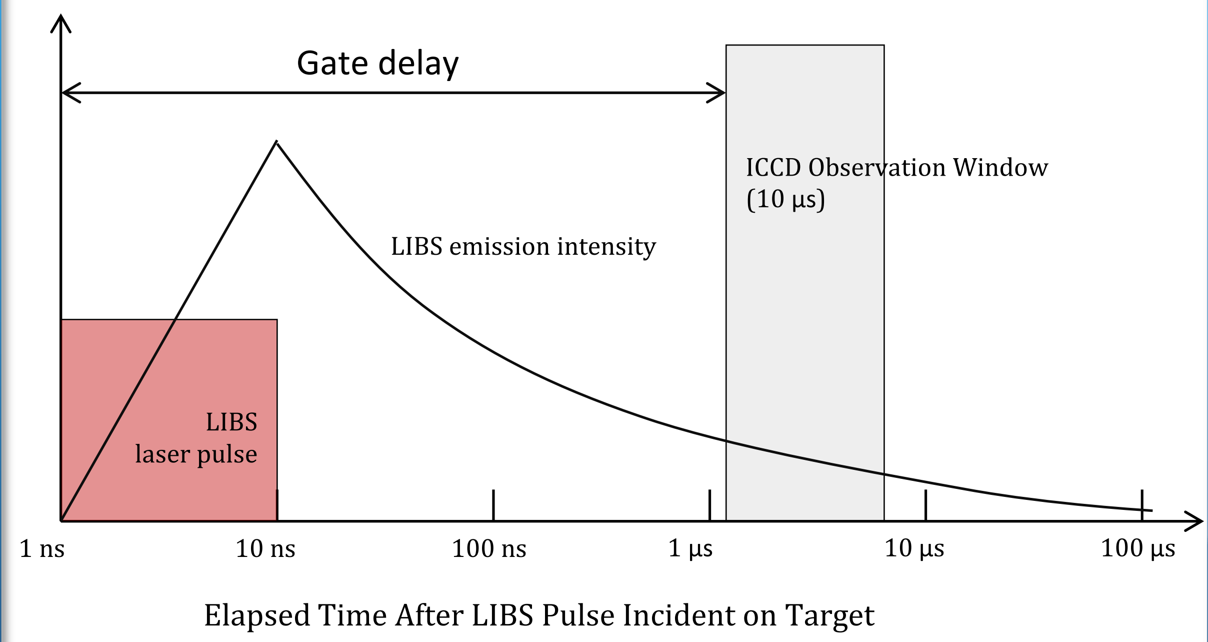


When we do a time-resolved spectroscopy of the plasma, we call it:

“Laser-induced breakdown spectroscopy”

or

LIBS



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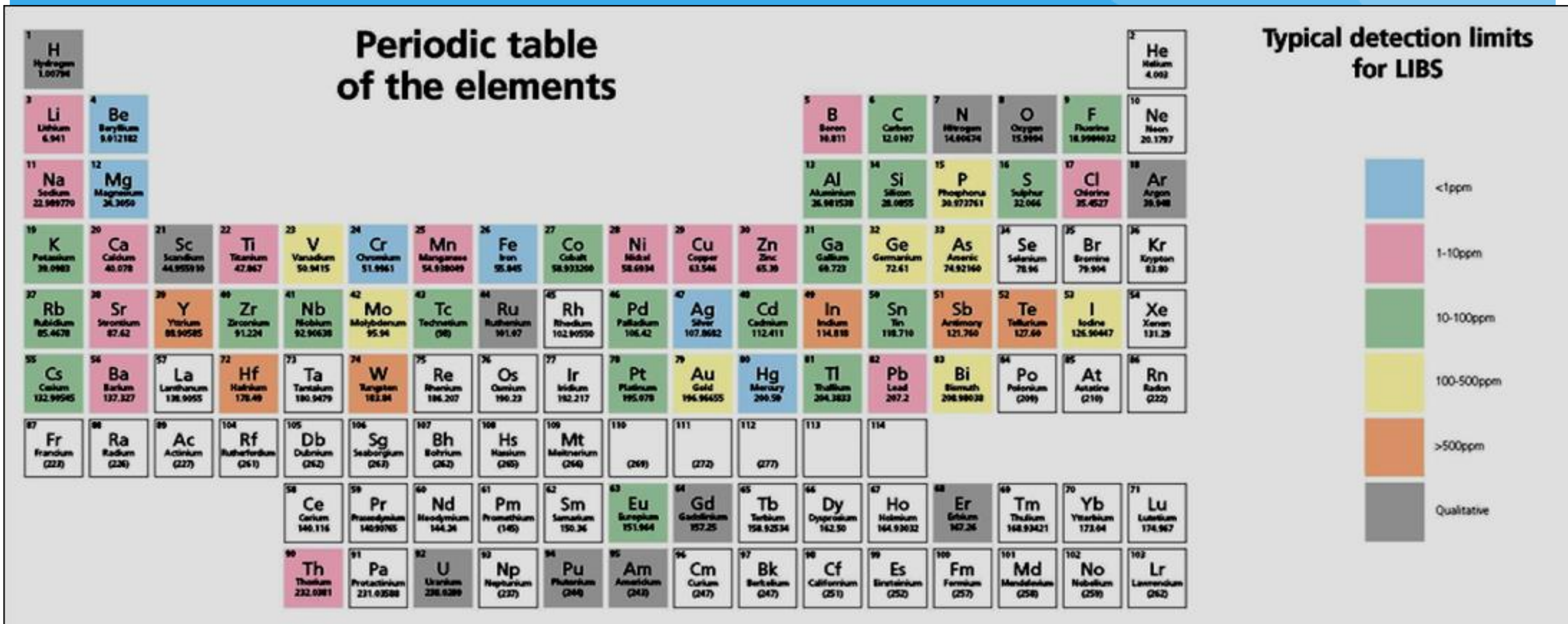
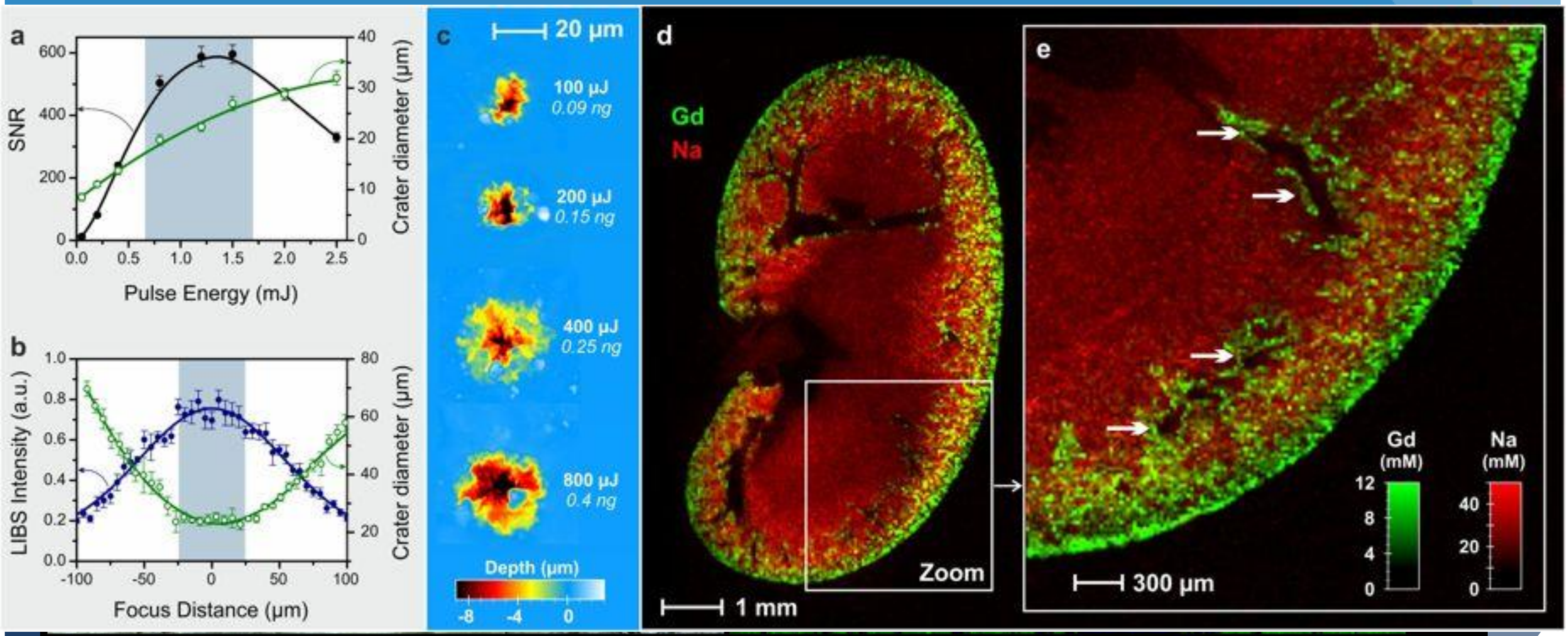
Advantages of LIBS – sensitivity across the periodic table



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

Advantages of LIBS - spatial resolution

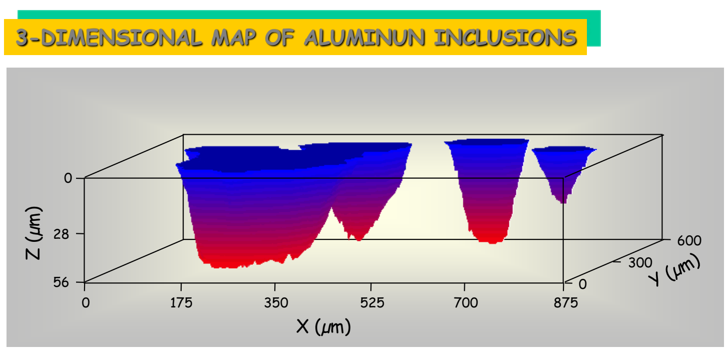
- Laser allows point sampling (1-100 micron)
- Elemental "surface maps" can then be created



Vincent Motto-Ros et al.

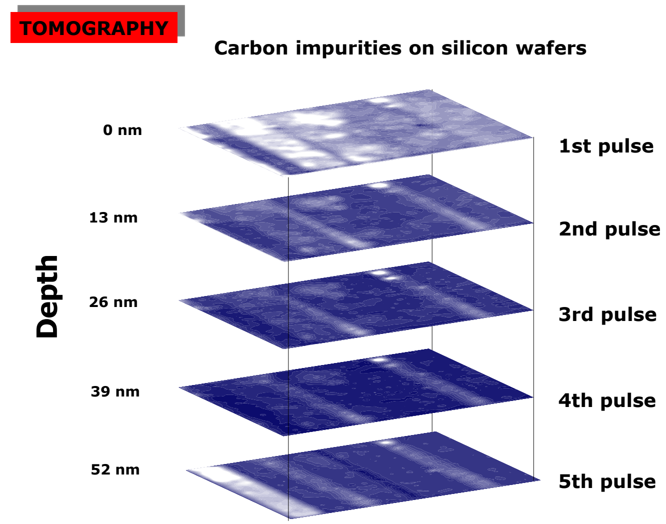
Advantages of LIBS - depth profiling

- Because laser only removes µg to ng of material, ablation crater only microns deep
- Subsequent shots thus sample progressively deeper layers



Courtesy of Ben Smith

- 13 positions
- 100 laser shots in depth
- Mapped volume = 600 x 875 x 56 µm3
- Repetition rate = 10 Hz
- Analysis time = 2 min and 30 s

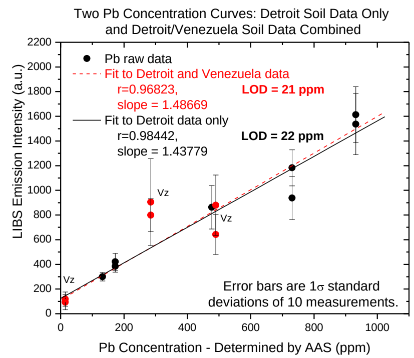


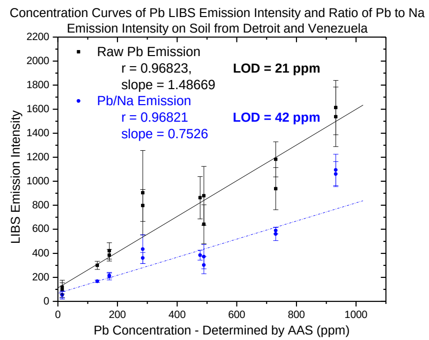
Nitrogen laser; Lateral resolution 15 µm, sampling depth 13 nm

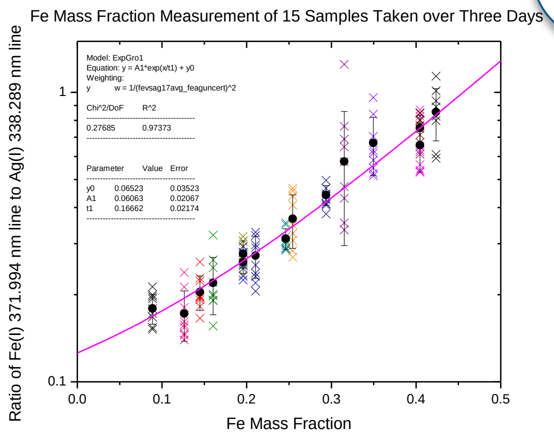
Courtesy of Ben Smith

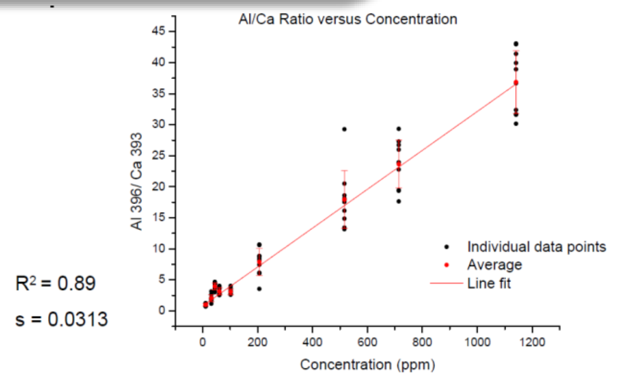
Advantages of LIBS – sensitivity & speed

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









Advantages of LIBS - CBRNE prototypes have been built

ad's-up display

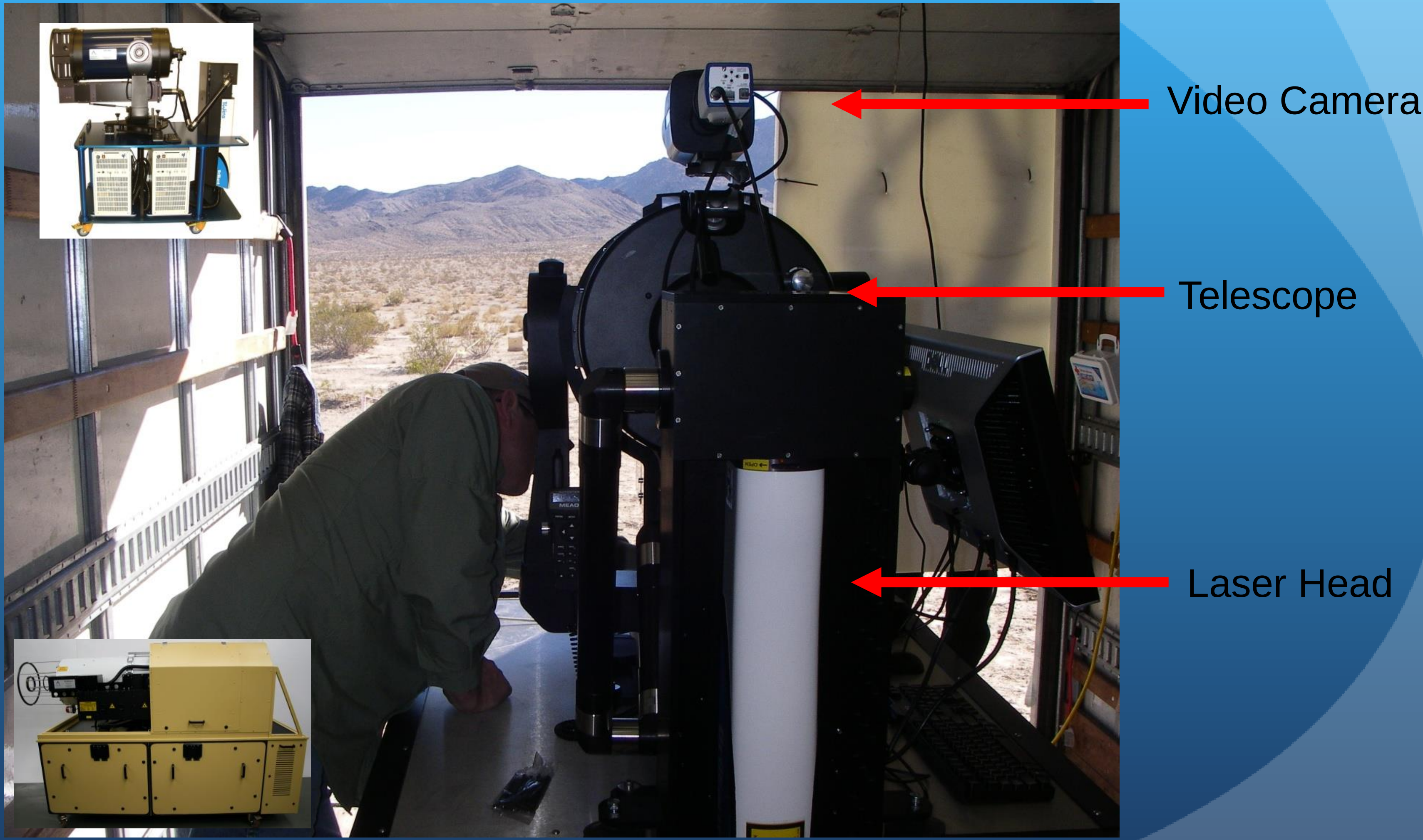
Backpack contai
broadband high-
resolution
spectrometer, las
power supply,
computer, and ba

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

Microplasma/
LIBS Event

courtesy of Ocean Optics.

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



Courtesy of A.J. Miziolek, A. Whitehouse

Advantages of LIBS - Commercial benchtop systems have been built

J200 – Applied Spectra

ChemReveal LIBS Desktop Elemental Analyzer – TSI

Advantages of LIBS – hand-held

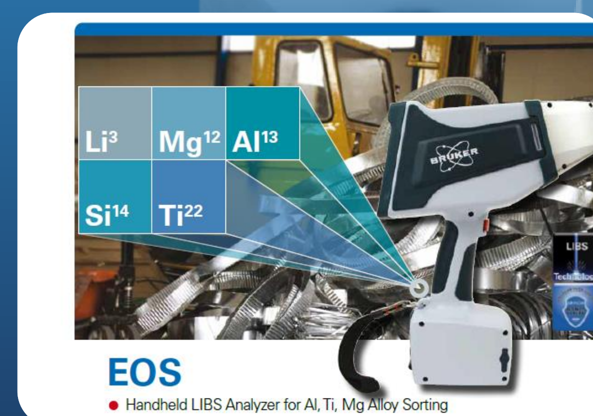
mPulse – Oxford Instruments

LIBZ – SciApps, Inc

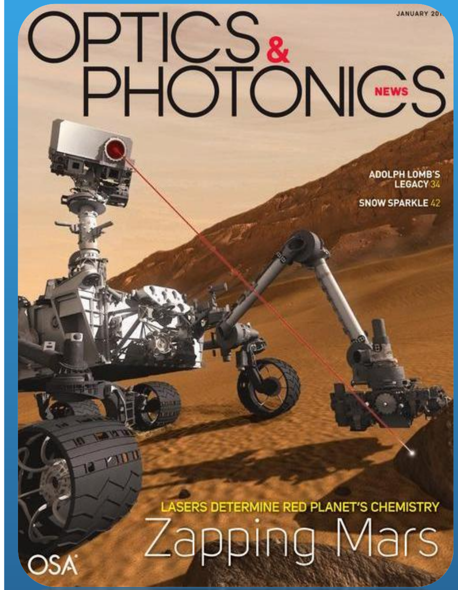
NanoLIBS – B&WTek

ChemLite- TSI, Inc

EOS500 - Bruker



Advantages of LIBS – Robustness/Up-time



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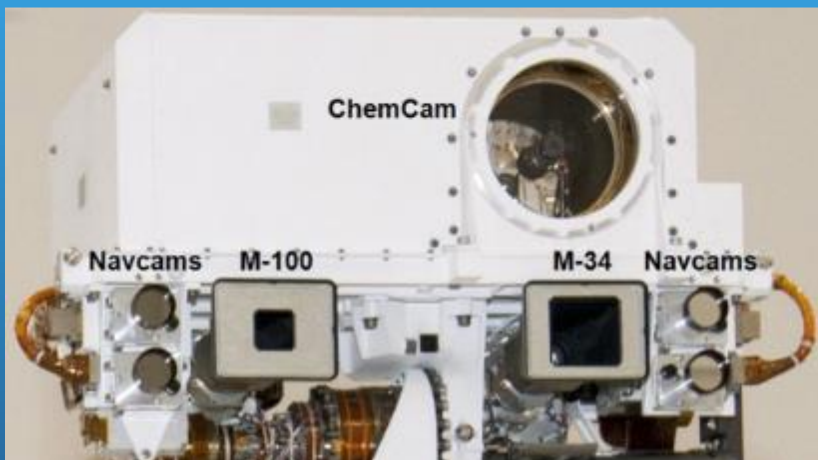

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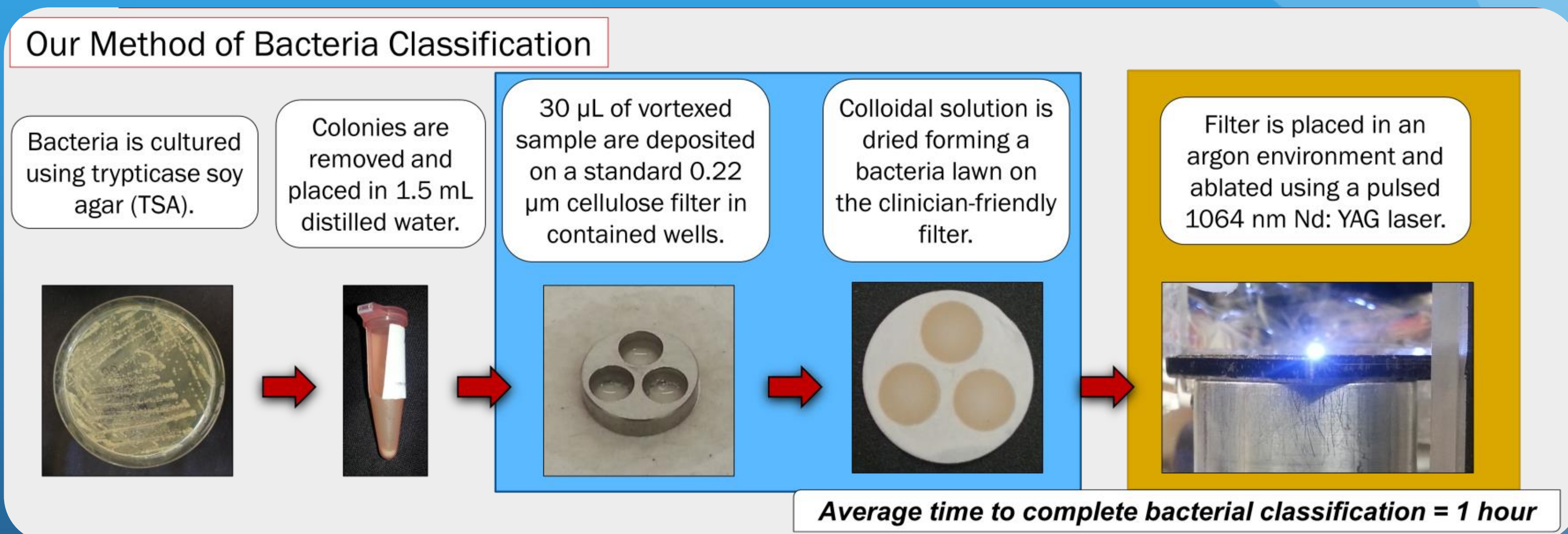
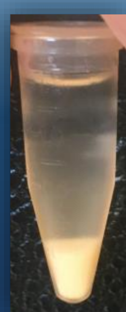
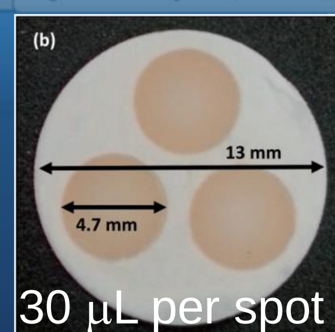
Rapid pathogen identification



$>10^9$ cfu/ml

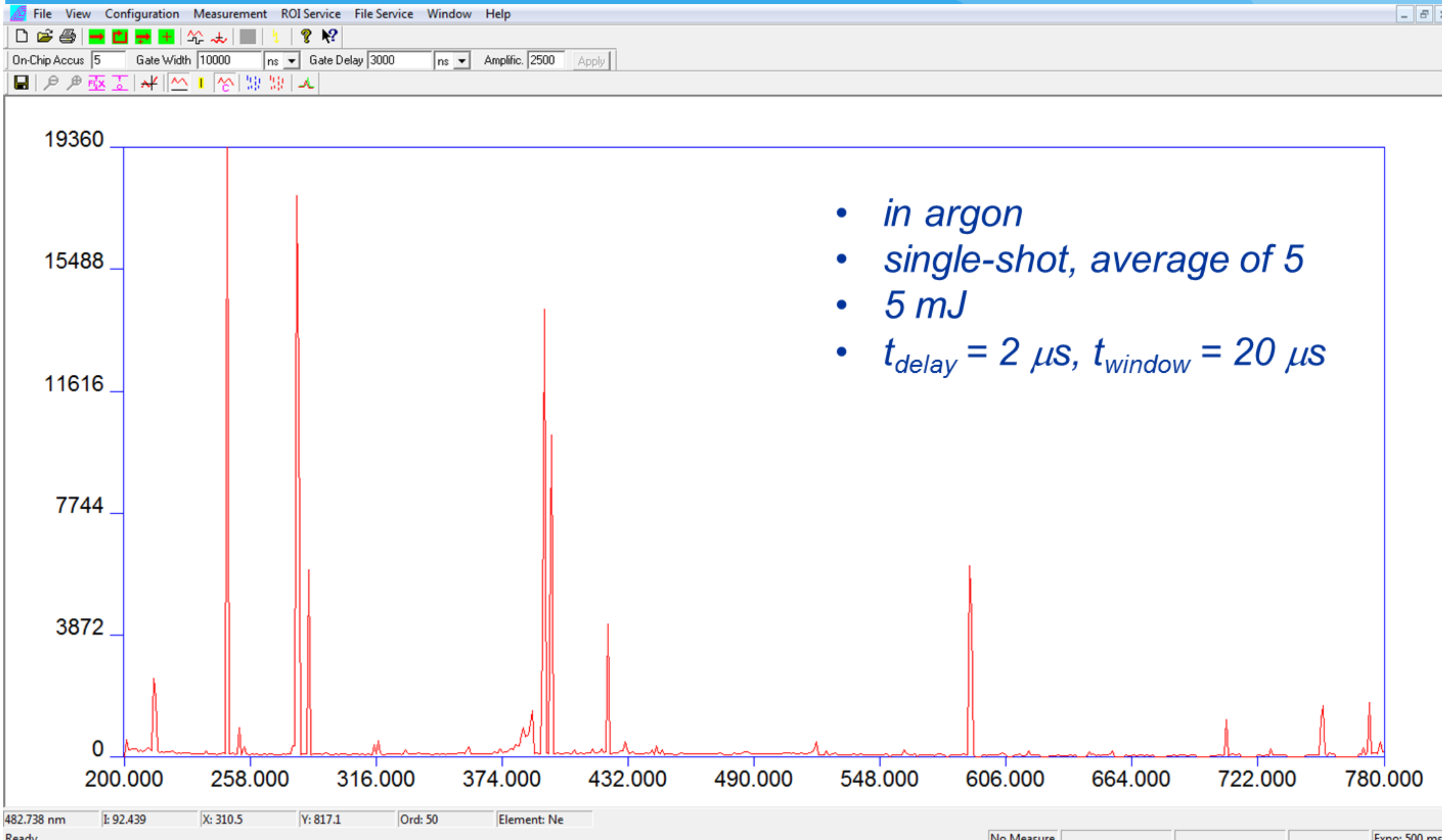




This is a LOT of bacteria!

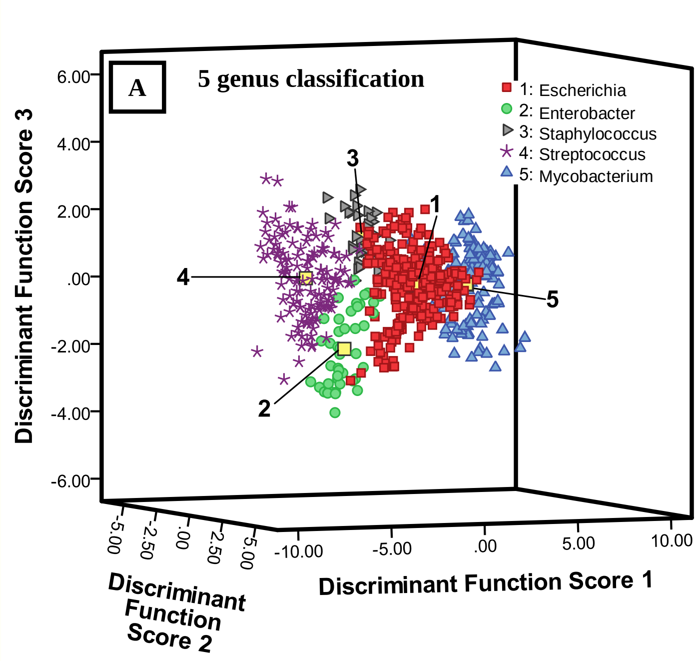
2014-2016

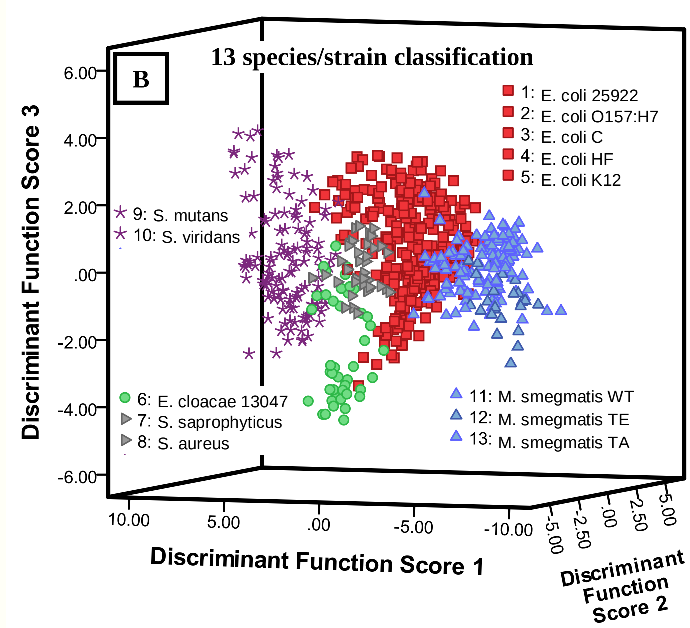
Variable Down Selection



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

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%

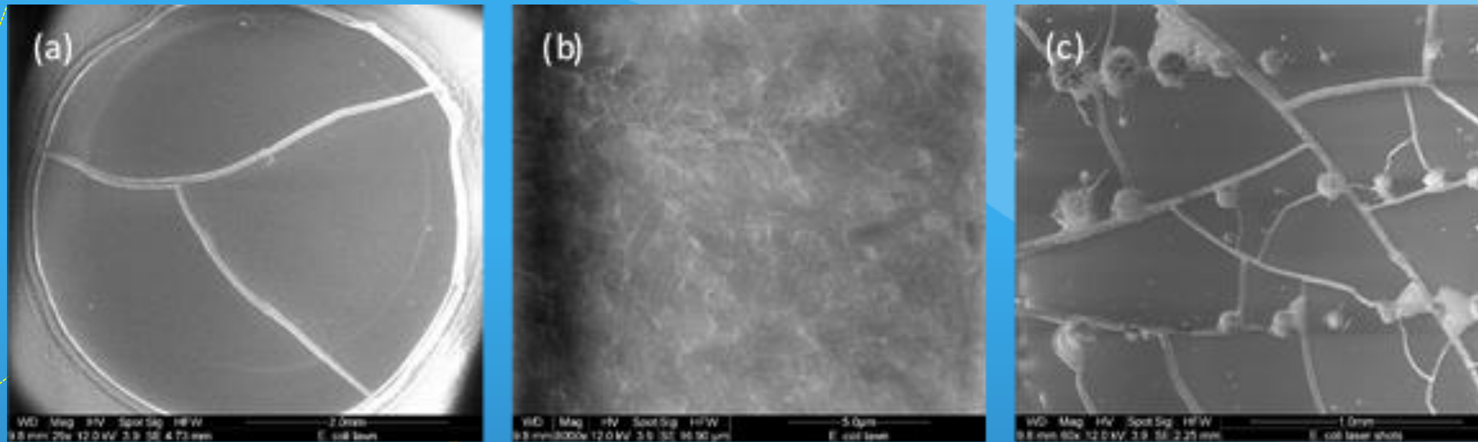
STAPHYLOCOCCUS	True	False
Positive	86.49%	5.85%
Negative	94.15%	13.51%

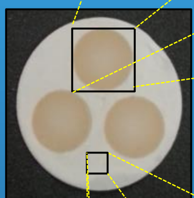
STREPTOCOCCUS	True	False
Positive	99.26%	13.32%
Negative	88.68%	0.74%

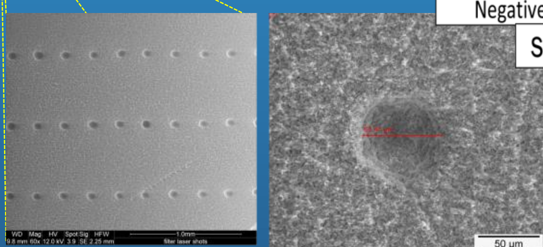
MYCOBACTERIUM	True	False
Positive	96.10%	4.08%
Negative	95.92%	3.90%

DFA: *Sensitivity*: 91.37 ± 16.39 % *Specificity*: 97.46 ± 9.35 %

PLSDA: *Sensitivity*: 93.13 ± 10.25 % *Specificity*: 90.60 ± 21.33 %







(a) DFA

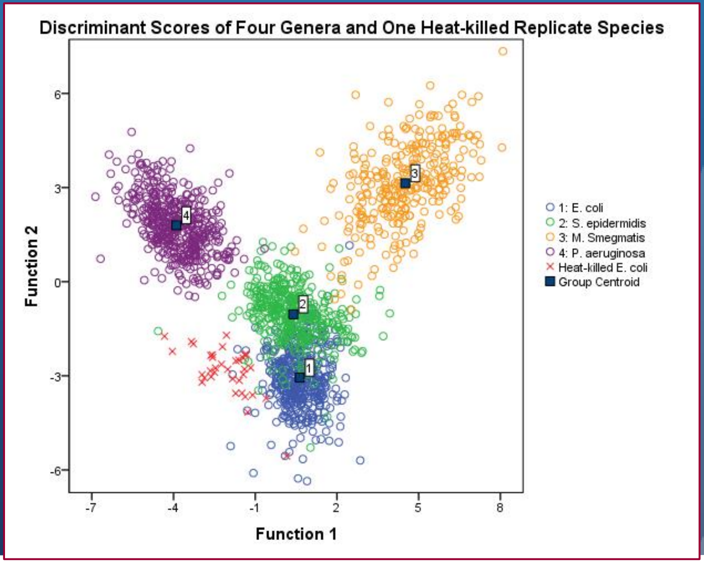
Escherichia	TRUE	FALSE	*Staphylococcus*	TRUE	FALSE
Positive	98.28%	0.77%	Positive	97.75%	1.44%
Negative	99.23%	1.72%	Negative	98.56%	2.25%
Mycobacterium	TRUE	FALSE	*Pseudomonas*	TRUE	FALSE
Positive	95.36%	0.33%	Positive	99.57%	0.22%
Negative	99.67%	4.64%	Negative	99.78%	0.43%

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

(b) PLS-DA

Escherichia	TRUE	FALSE	*Staphylococcus*	TRUE	FALSE
Positive	96.55%	1.12%	Positive	96.75%	1.53%
Negative	98.88%	3.45%	Negative	98.47%	3.25%
Mycobacterium	TRUE	FALSE	*Pseudomonas*	TRUE	FALSE
Positive	97.02%	0.41%	Positive	98.92%	0.33%
Negative	99.59%	2.98%	Negative	99.67%	1.08%

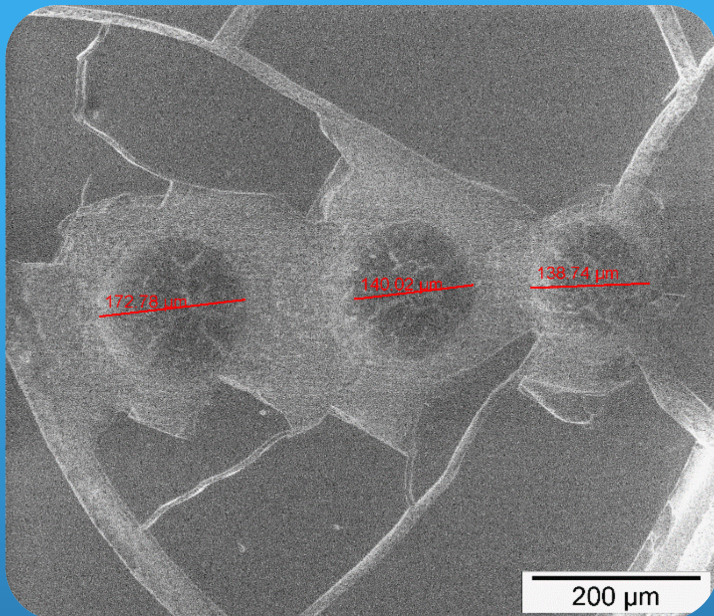
Sensitivity: 97 ± 3% Specificity: 99 ± 2%



Highly efficient discrimination still possible on nitrocellulose medium

DFA and PLS-DA perform similarly

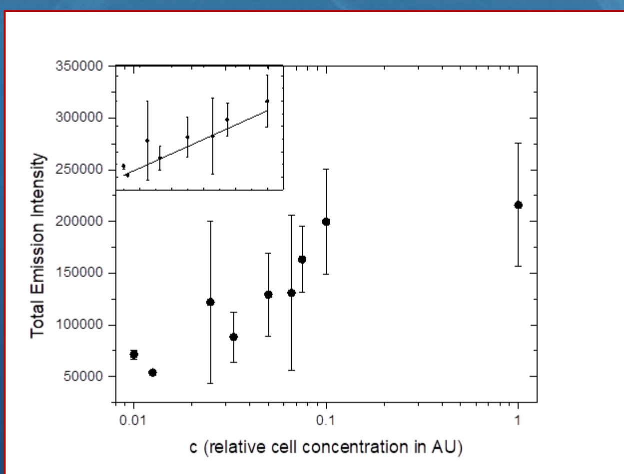
2014-2016

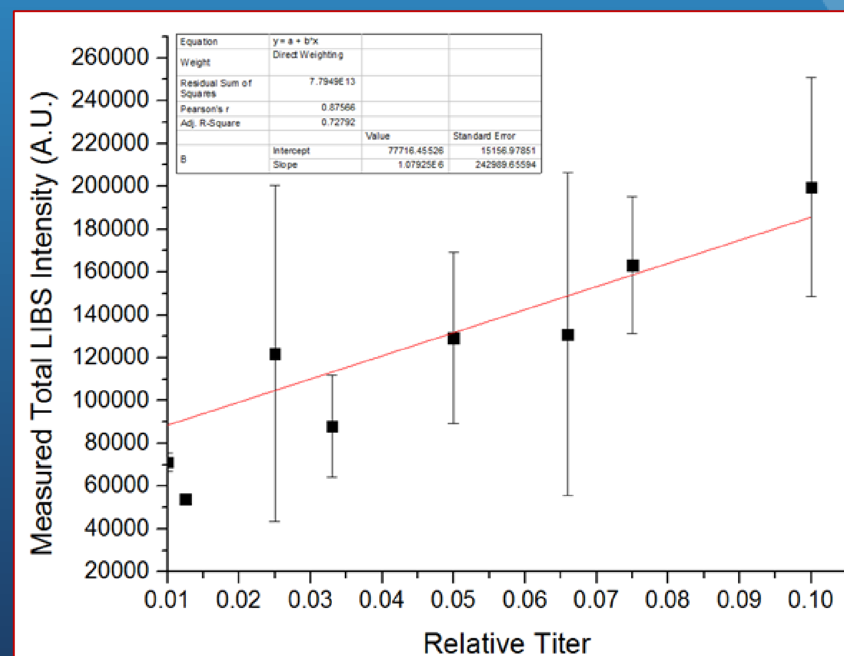


- ✓ Know ablation area
- ✓ Know bacterial titer (from absorption optical densitometry)
- ✓ Know bacterial deposition area

➢ **Known # cells per ablation spot**

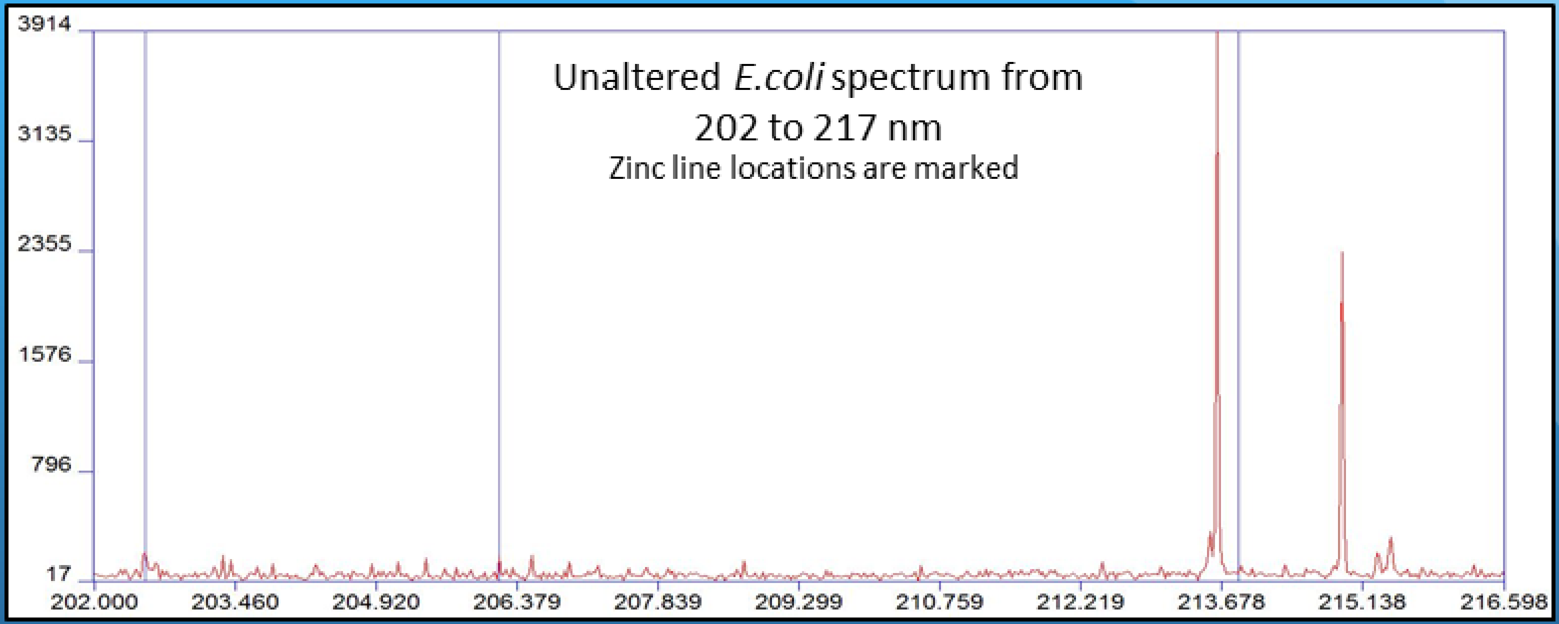
limit of detection of 48000±12000 CFU per ablation event





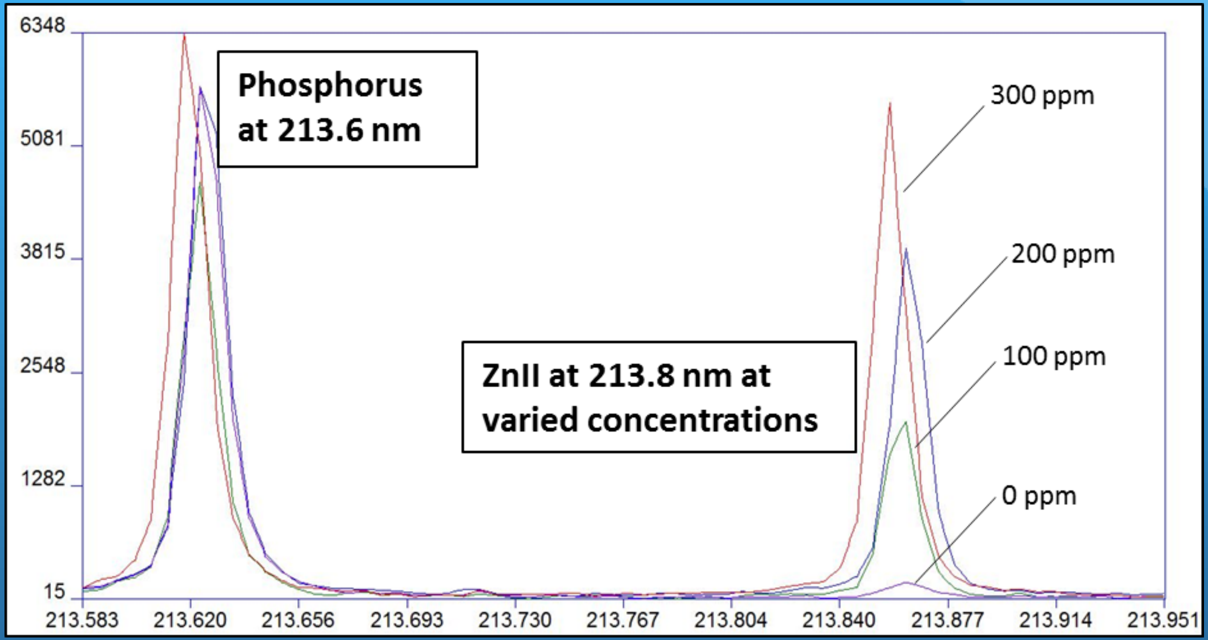
2014-2016

Altering Cell Metal Content: Zinc



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

Altering Cell Metal Content: Zinc



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

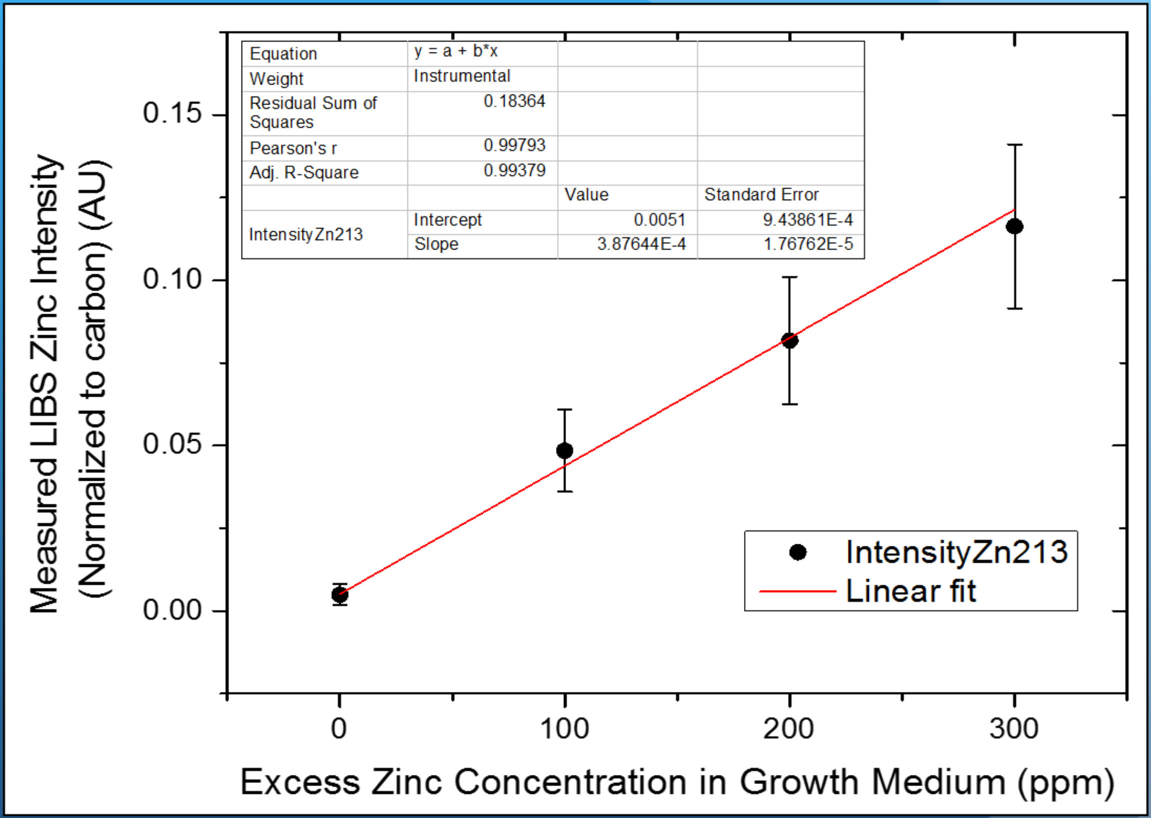
2016

Altering Cell Metal Content: Zinc

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

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

The maximum concentration allowable for drinking water is 5 ppm.

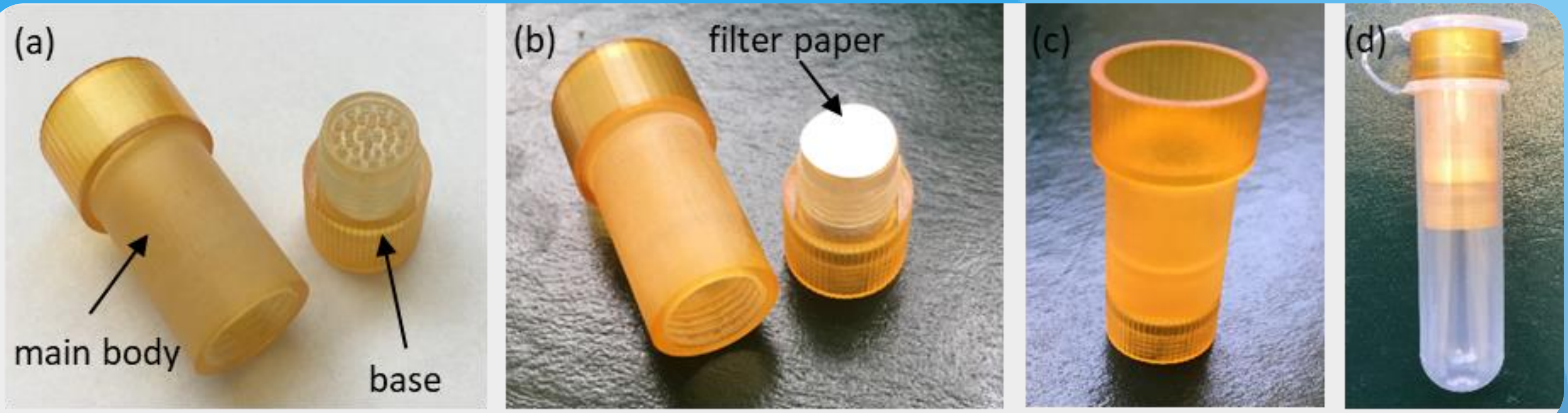


Environmental Application

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

2016

New Mounting Procedure: Concentration by Centrifugation



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

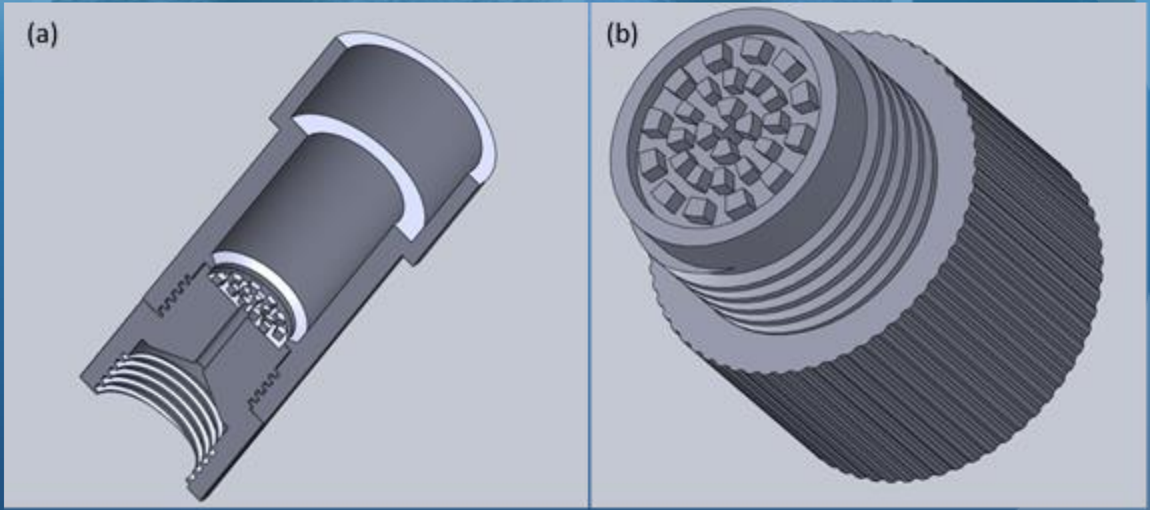
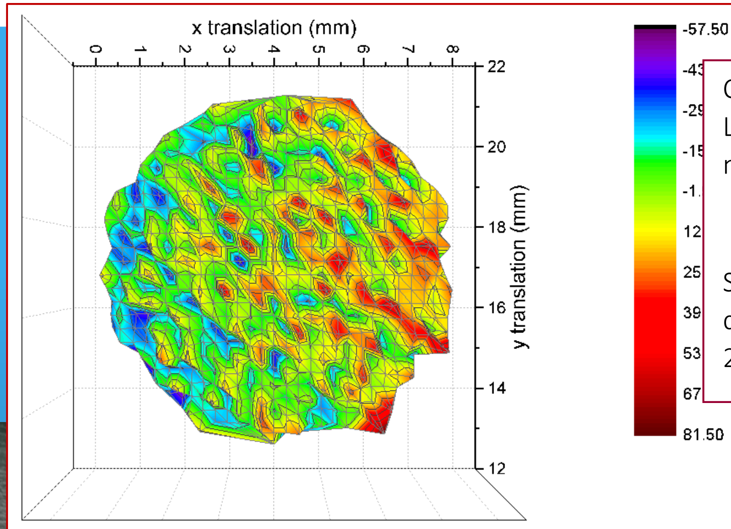


Figure 6.1: (a) Full centrifuge insert design in cross section. Filter paper is placed on the male end (b) of the device, and a seal is produced by the pressure generated by the threads. Pedestals under the filter paper prevent it from resting directly on a flat surface, allowing water to freely pass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

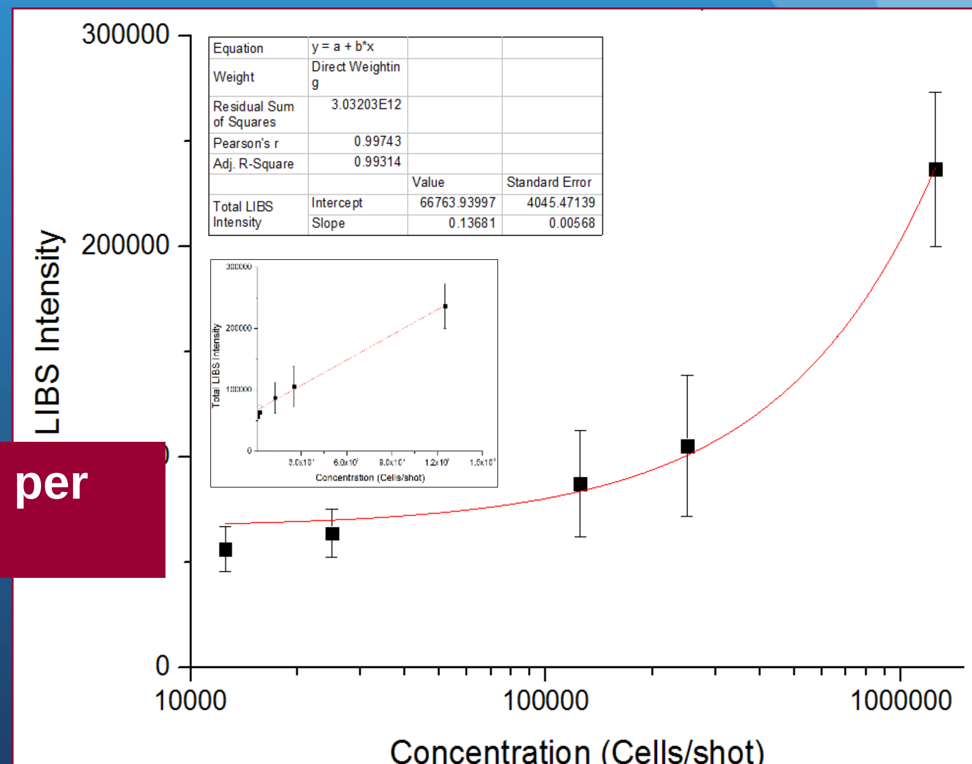
2016-2018



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

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

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

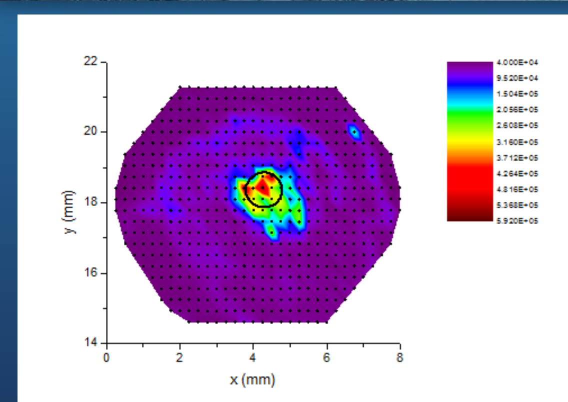


limit of detection of 60000±5000 CFU per ablation event

2016

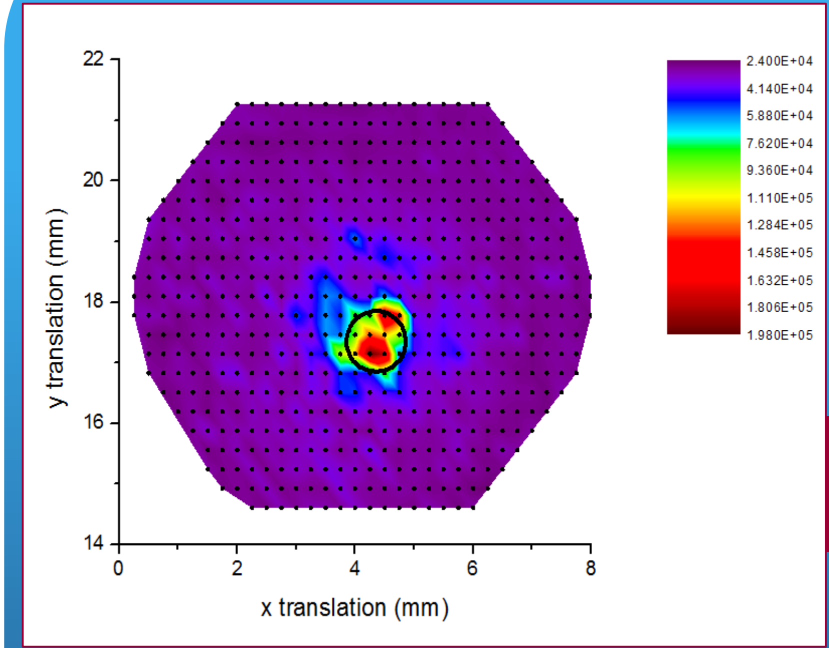
New Mounting Procedure: Concentration by Cone

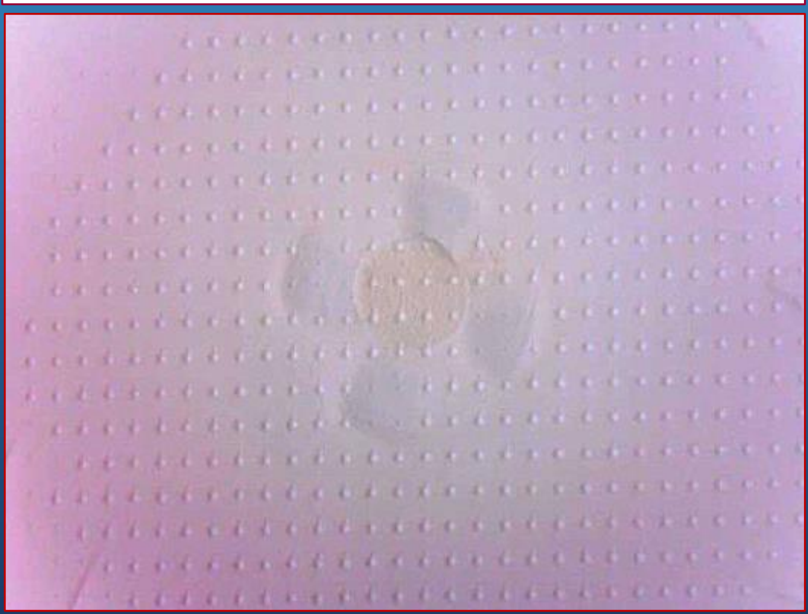
To concentrate all the bacteria into one spot (one laser shot) a custom funnel was constructed for our centrifuge insert

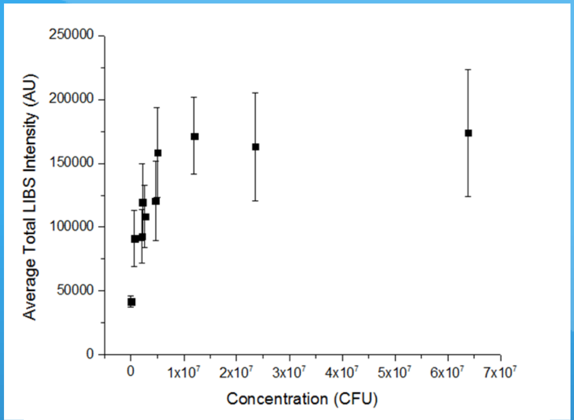


Each point on the map corresponds to a single laser shot, and the color indicates the LIBS bacterial intensity, with purple indicating no LIBS bacterial signal, and red indicating the region with the strongest LIBS bacterial signal.

2017-2018

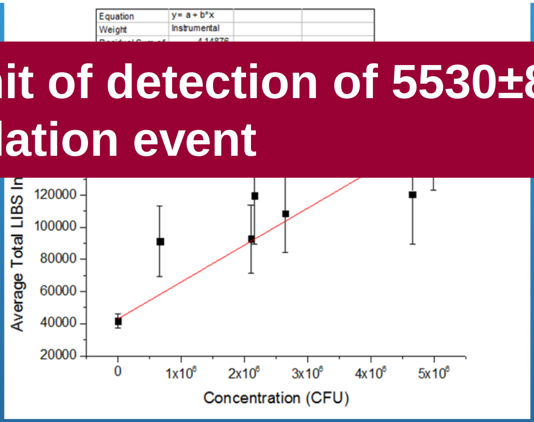




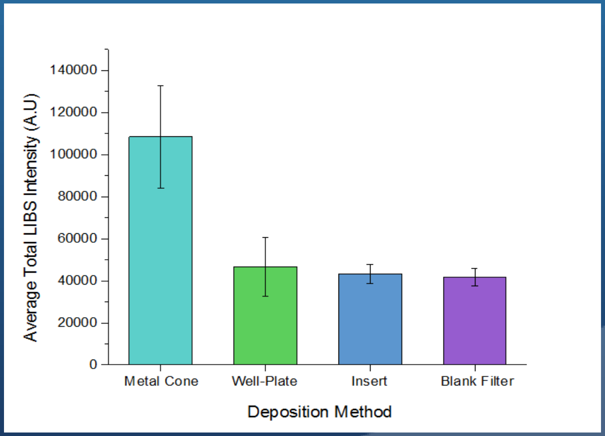


Concentration curve

limit of detection of 5530±872 CFU per ablation event

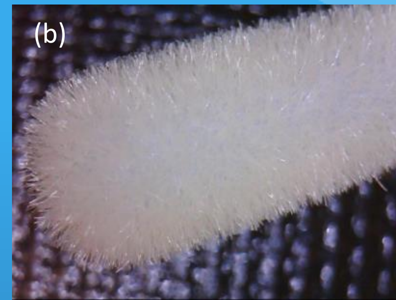


Linear region of concentration curve

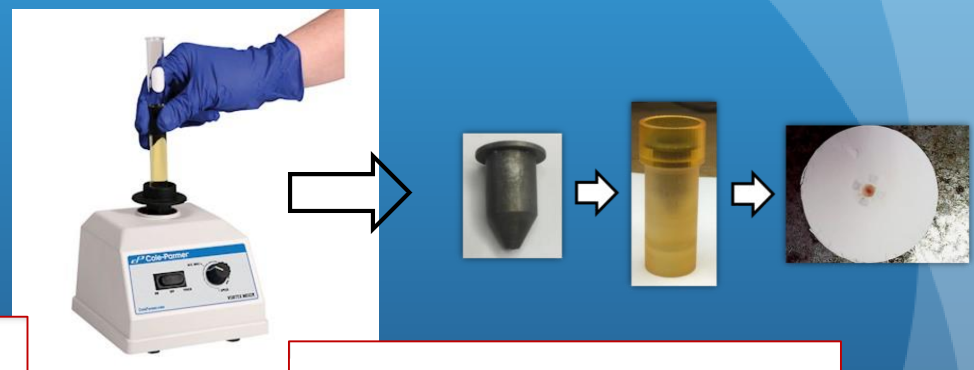


Bacteria detected when other methods could not

New Collection Procedure: Swabs

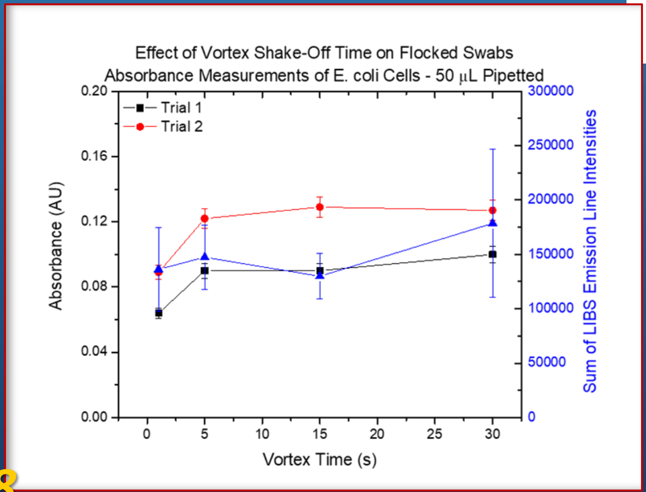


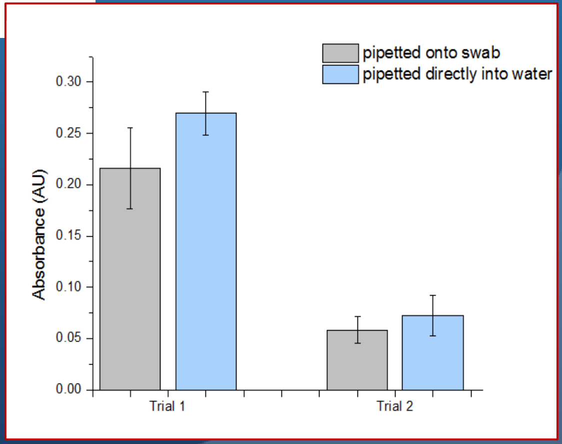
(a) Flocked swab used in this work. (b) Flocked swab zoomed-in on the tip



Cannot shoot right on the swab

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





2017-2018

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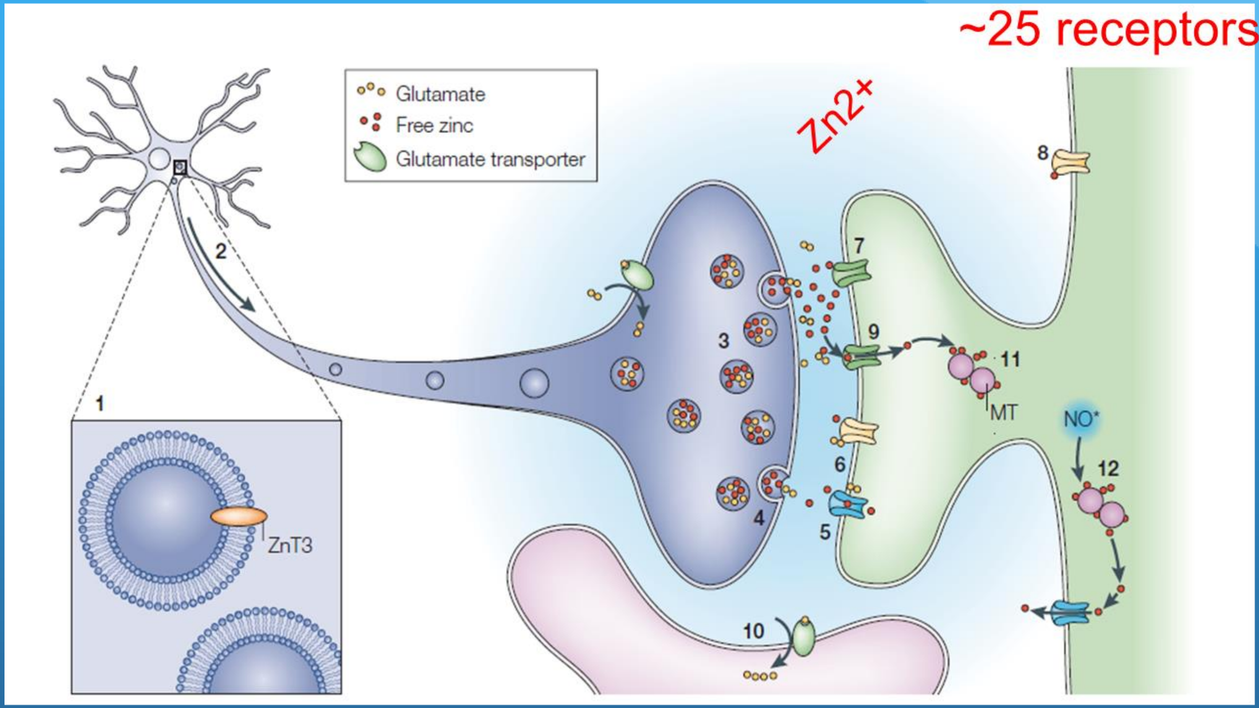
3. Biomedical Applications of LIBS
   a. A new paradigm for rapid pathogen identification
   b. A real time assay for nutritional zinc deficiency
   c. An ecological tool for analyzing fish otoliths

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?

Line in the

represent t ve nc ntratio the body.

But we need a rea time biomedical assay

Fingernail Structure

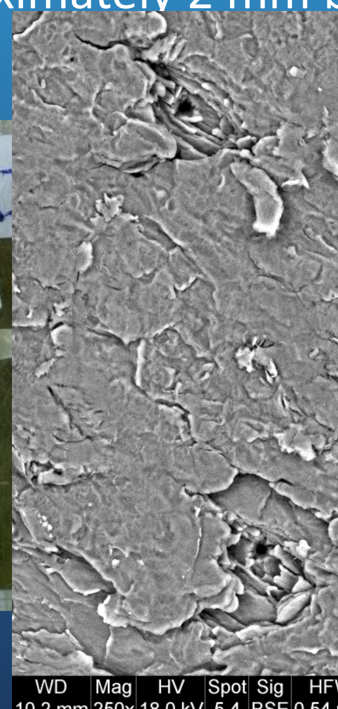
Although they <u>look</u> like this…

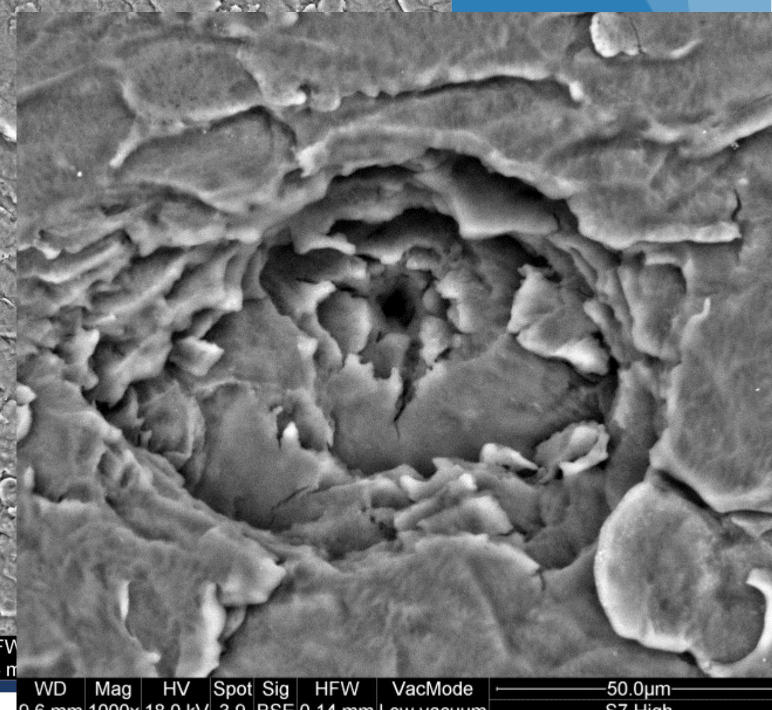
…they are actually like this!

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

Preparation of Nails

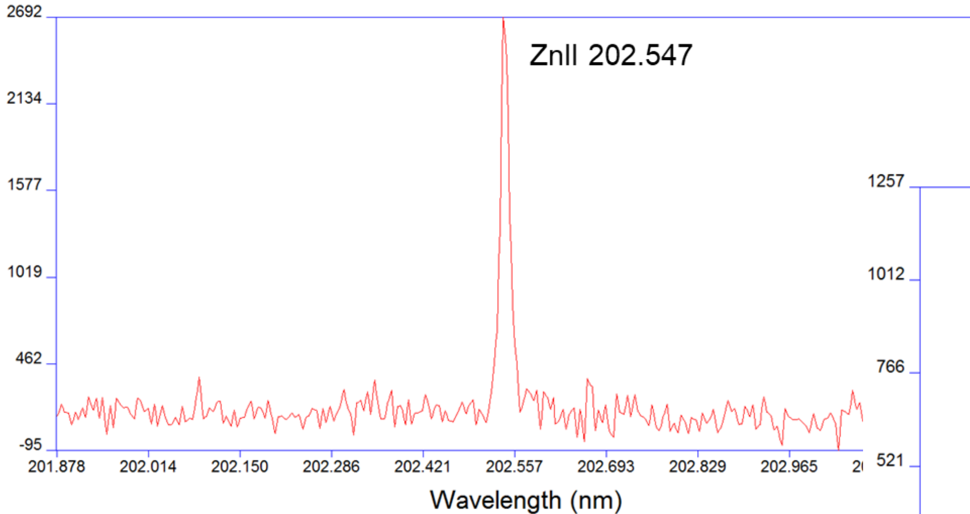
- Nail clippings of the index, middle and ring fingers (both right and left hands) of 5 subjects were taken→ a total of 6 nail clippings per subject.
- Clippings were cleaned with acetone in an ultrasound bath for 10 minutes and allowed to dry for 20-30 minutes.
- Clippings are cut into approximately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

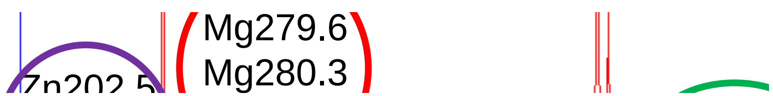


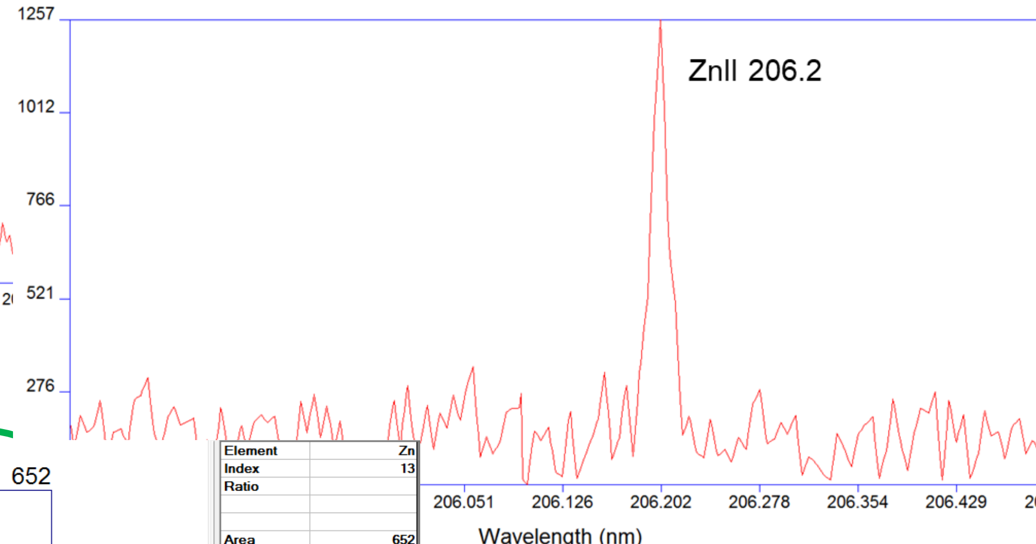


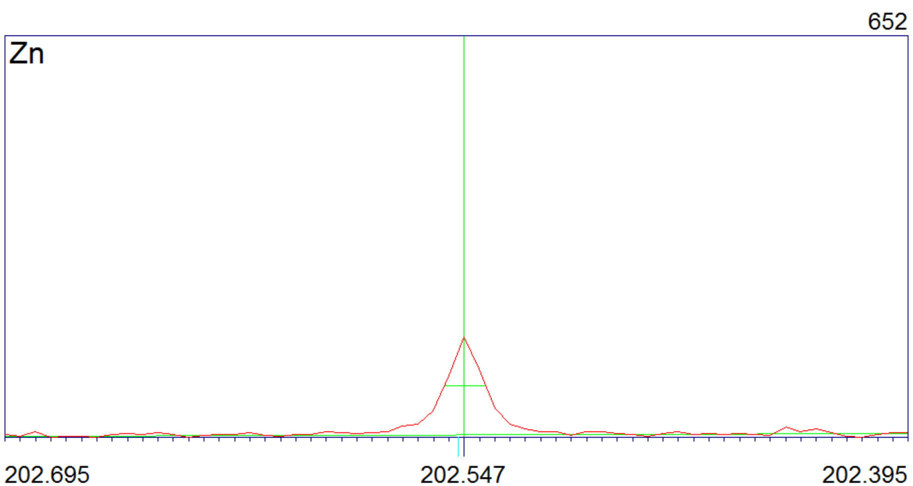
Zinc easily visible

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

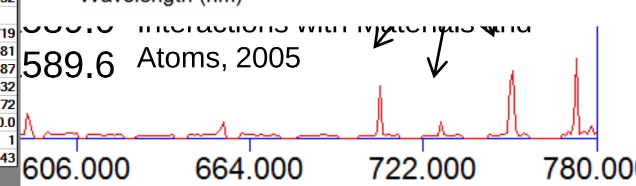




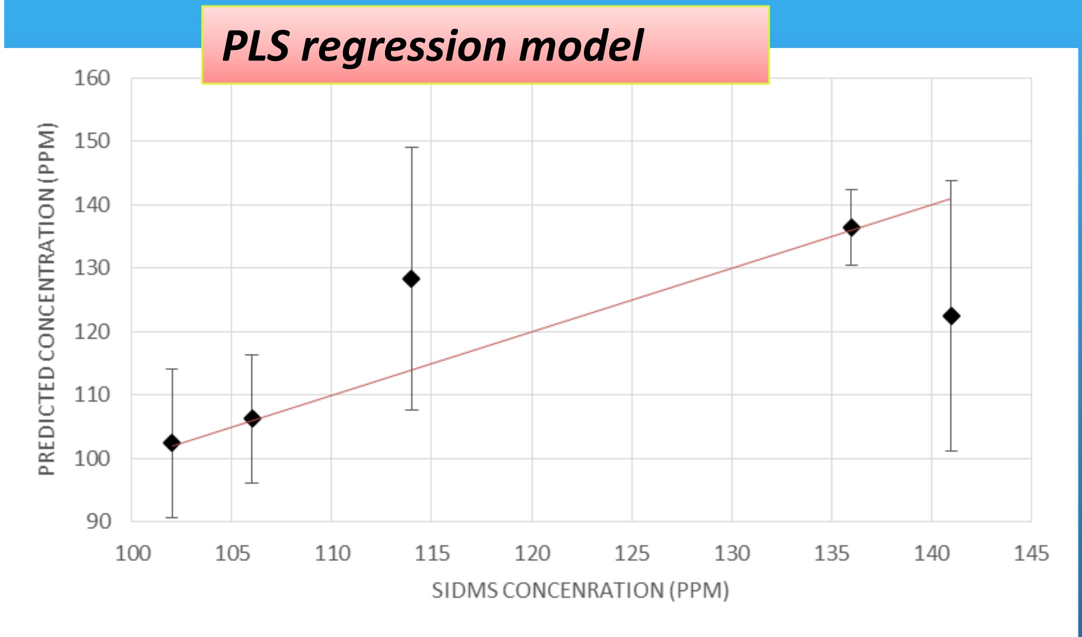




Element	Zn
Index	13
Ratio	
Area	652
Maximum	3719
Minimum	3381
Offset	3387
Height	332
Widht Half M.	2.72
Position	30.0
SC Fac	1
Stdev	6.43



Results



- 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 1σ 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

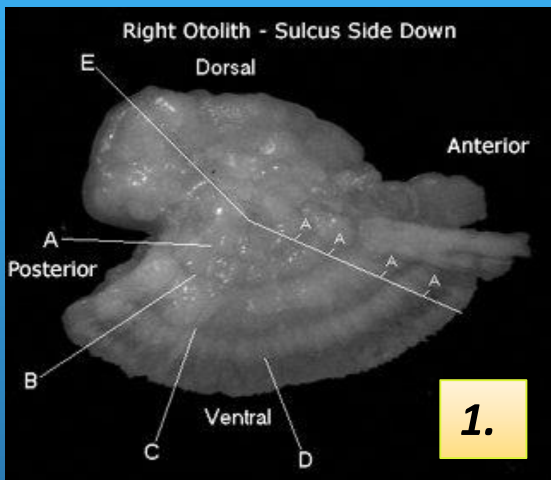


Image acquired from http://wgosm.npafc.org/MarkFAQ.asp

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

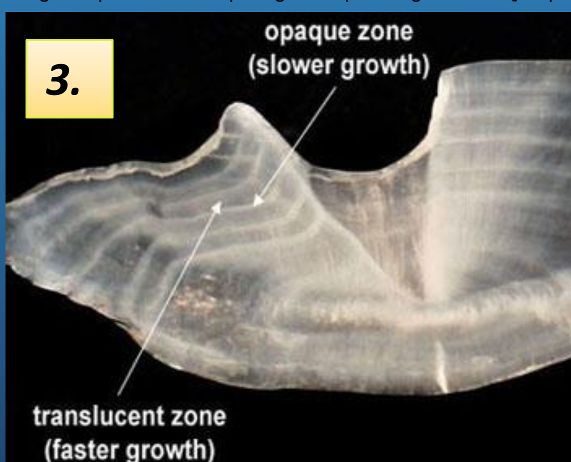
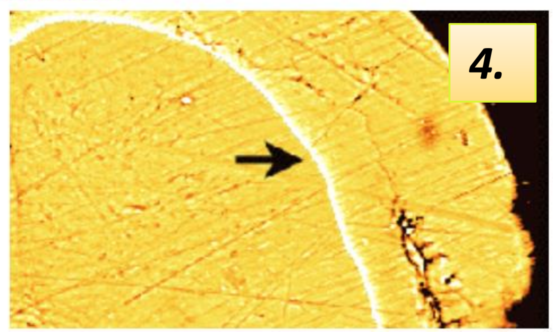


Image acquired from http://http://keywordsuggest.org

Strontium

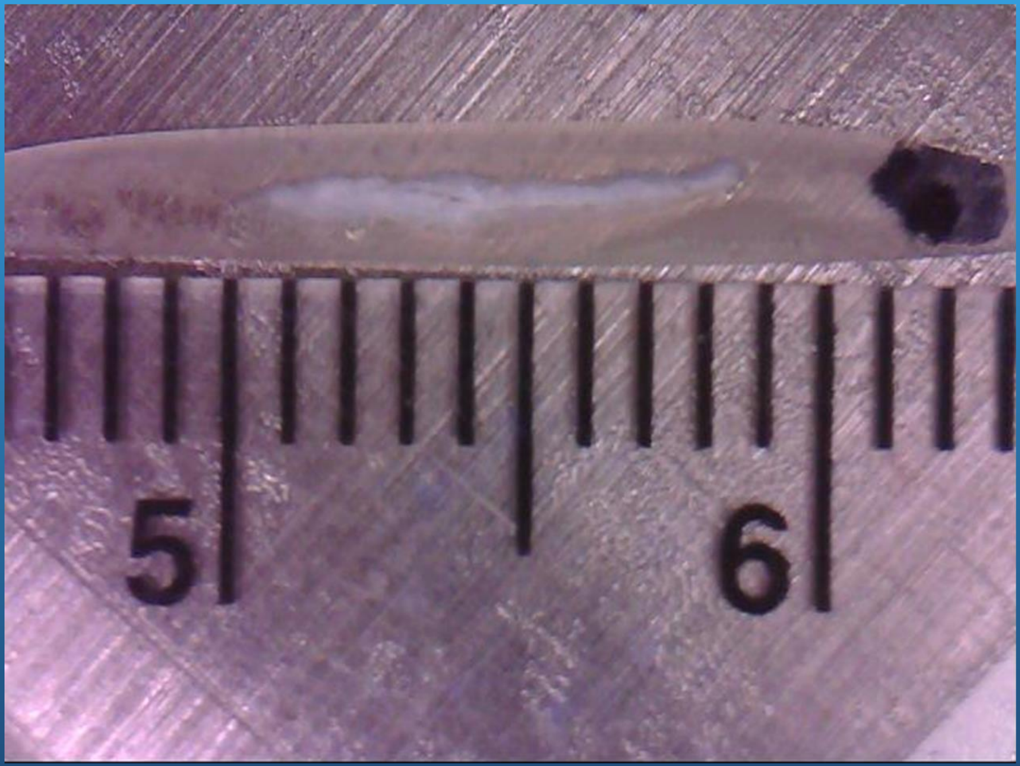


Salmonids can be successfully mass-marked using strontium chloride at any life history stage. Thermal marks, in contrast, can only be applied during a 2 to 4 week period after the eyes form in the embryos. One drawback of the strontium mark, however, is that they cannot be viewed using a traditional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

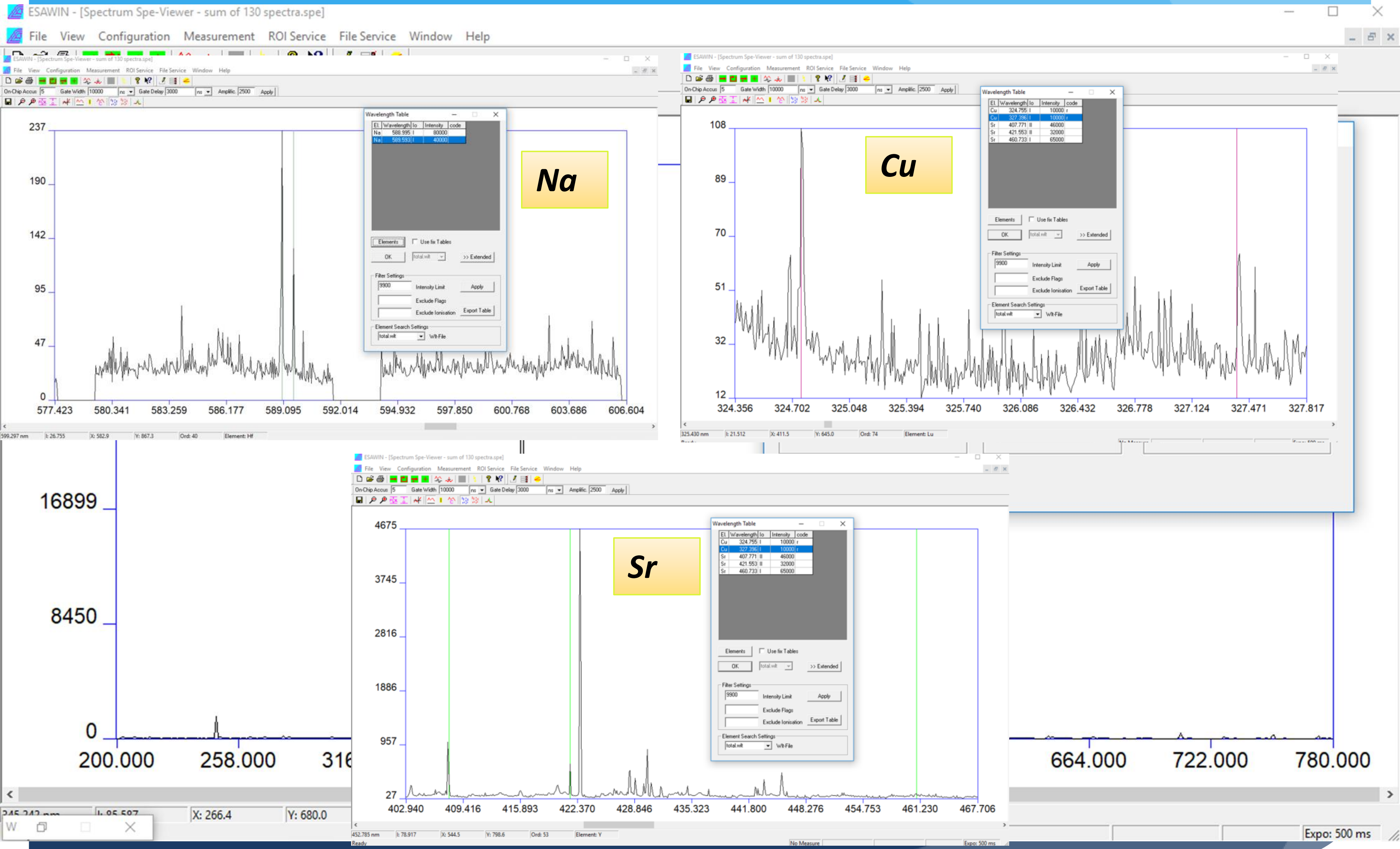
Can we monitor fresh/salt water migration via the elemental concentration?

Otolith samples prior to LIBS ablation

Mounted in a paraffin wax and cross-sectioned



LIBS Otolith Spectra



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All Credit to the Students!