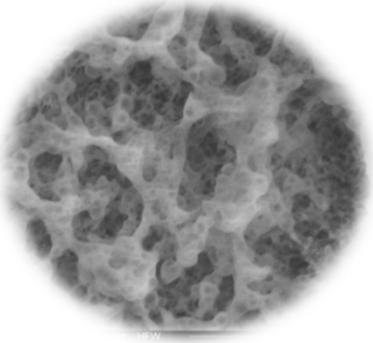
Biomedical and Biological Applications of Laser-Induced Breakdown Spectroscopy...



Steven J. Rehse

Alexandra E. Paulick

Dylan J. Malenfant

Christopher J.S. Heath

Paul Dubovan

Robert Valente

Naila Rahman

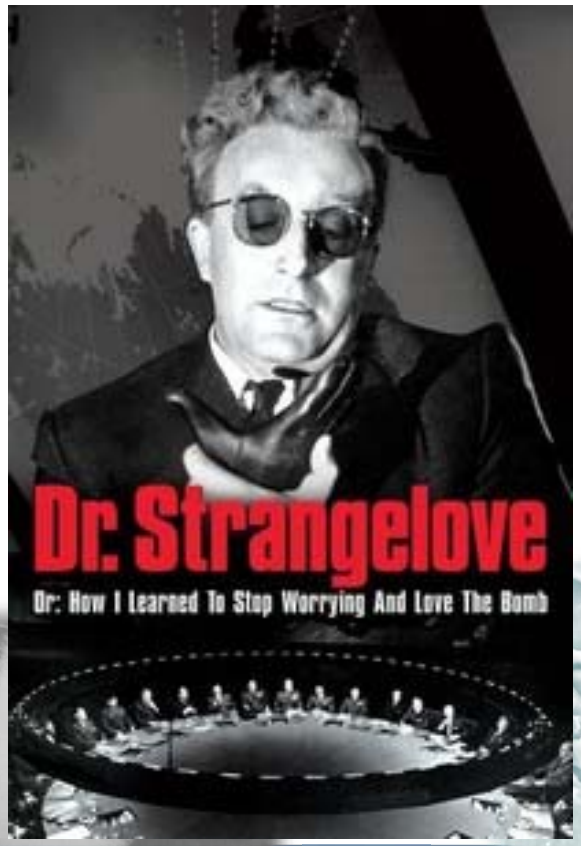
Vlora Riberdy

Anthony Piazza

University of Windsor

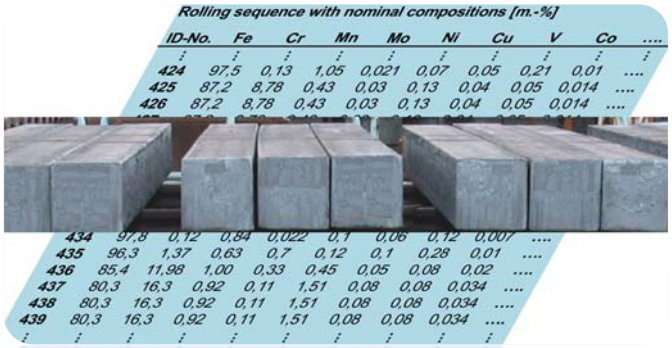
PITTCON CONFERENCE & EXPO 2018

…or: How I Learned to Stop Worrying and Love Shot-to-Shot Irreproducibility



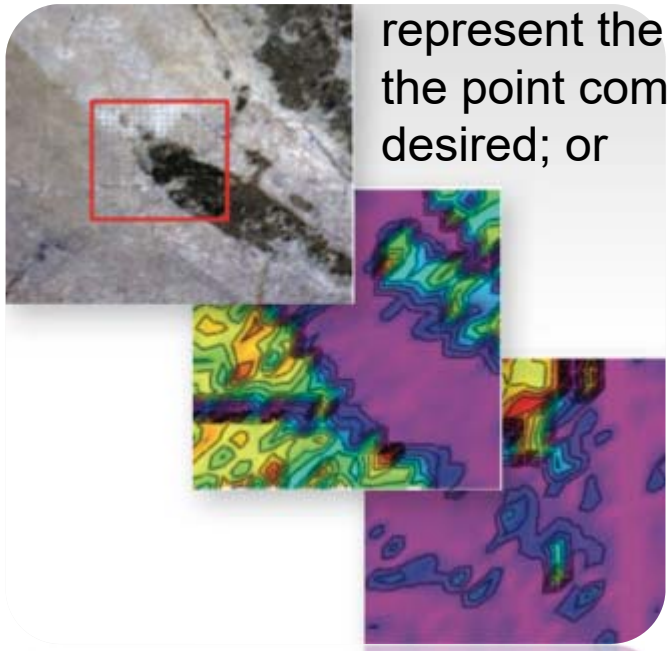
When we say that LIBS *"…requires little to no sample preparation…"* we usually mean:

The spot we are sampling is representative of the bulk; or



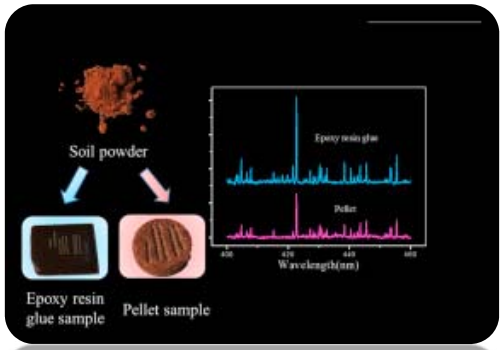
Sturm, Meinhardt, Fleige, Fricke-Begemann, Eisbach, "Fast identification of steel bloom composition at a rolling mill by laser-induced breakdown spectroscopy," SAB 136, 2017, 66-72

The spot does not represent the bulk, but the point composition is desired; or



Innovative Elemental Mapping of Geological Minerals with Applied Spectra's J200 Tandem LA-LIBS

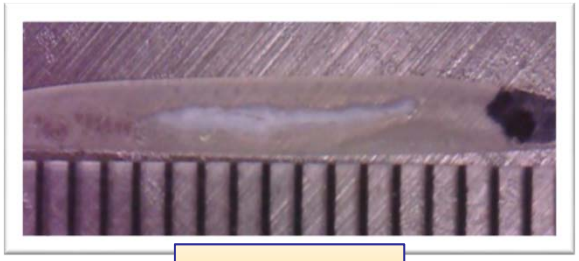
There is no bulk, but the sample has been homogenized to create a "pseudo-bulk."



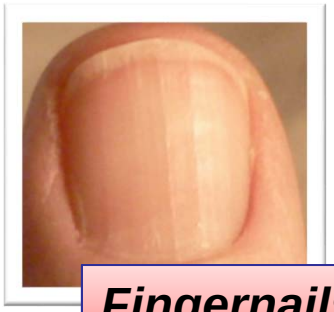
Shi, Lin, Duan, "A novel specimen-preparing method using epoxy resin as binding material for LIBS analysis of powder samples," Talanta 144, 2015, 1370-1376

But Biologicals / Biomedicals…?

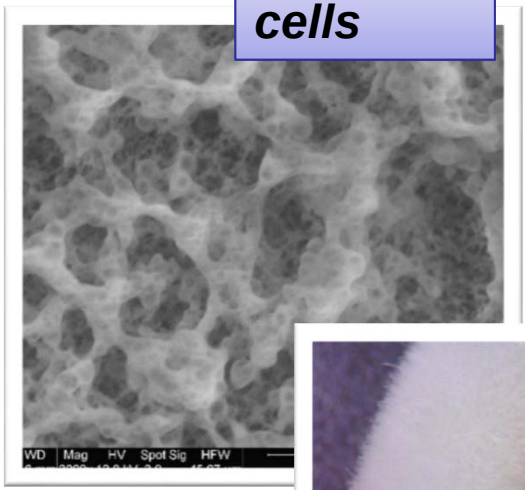
- Systems we have been investigating seem to be intrinsically structurally non-uniform (although homogeneous)
- Today, I'll be talking about several problematic systems:



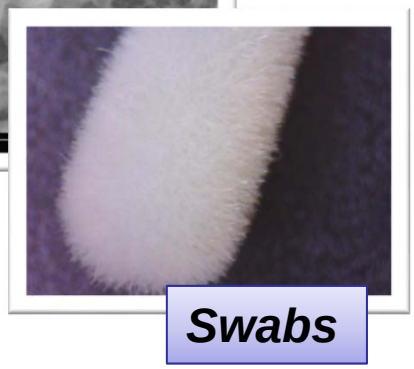
Otoliths



Fingernails

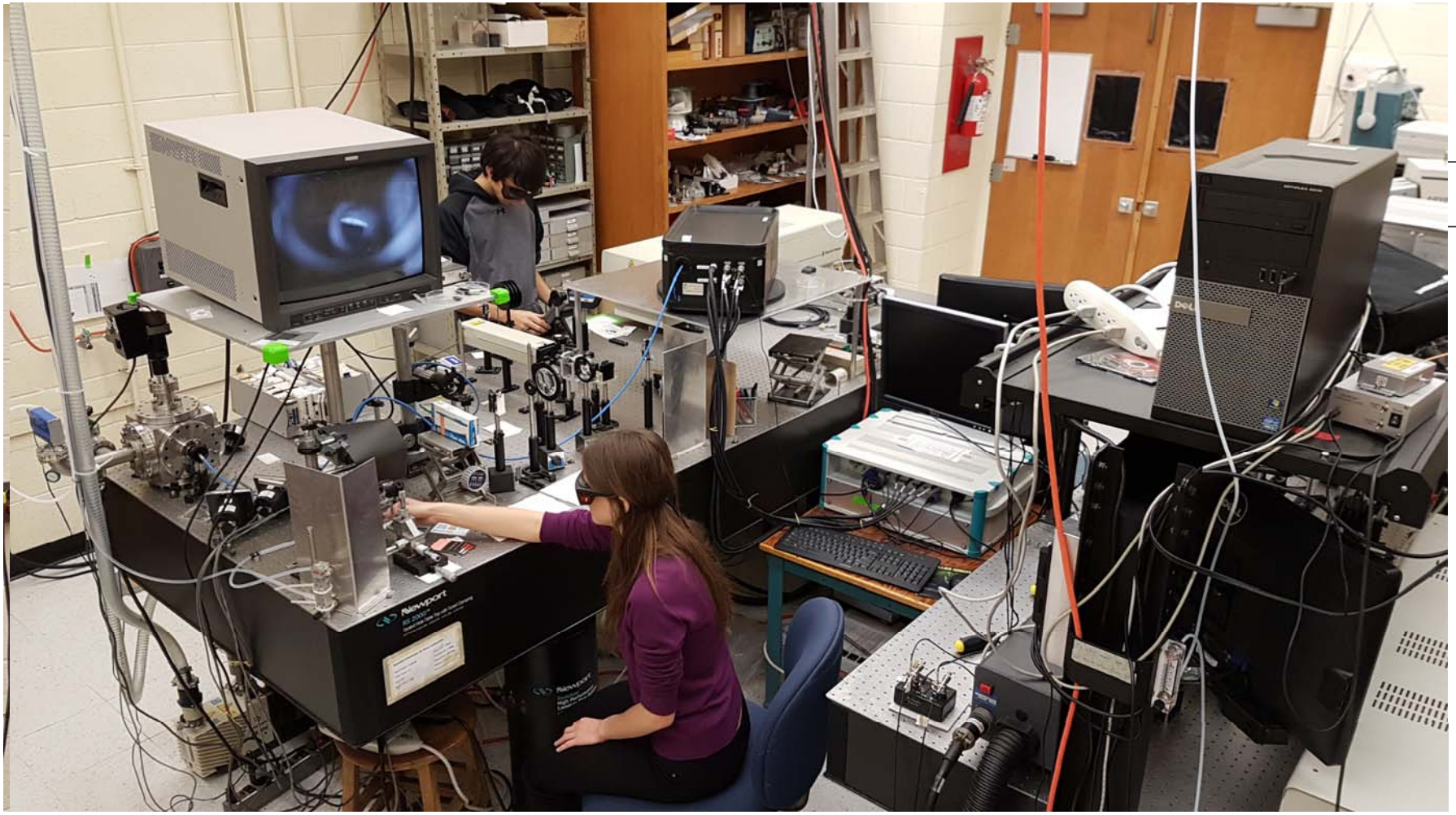


Bacterial cells



Swabs

All Experiments



4 Things to Know About Otoliths

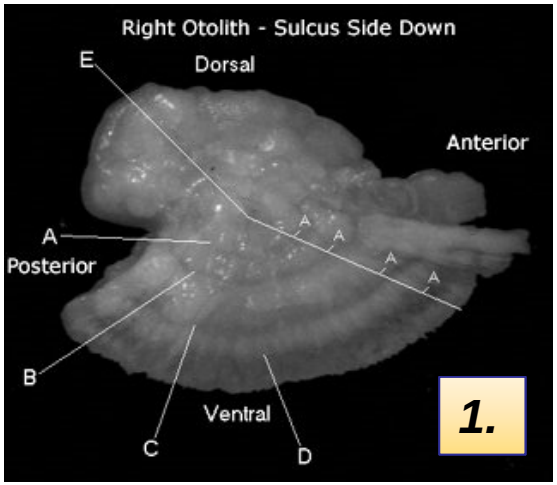


Image acquired from http://wgosm.npafc.org/MarkFAQ.asp

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

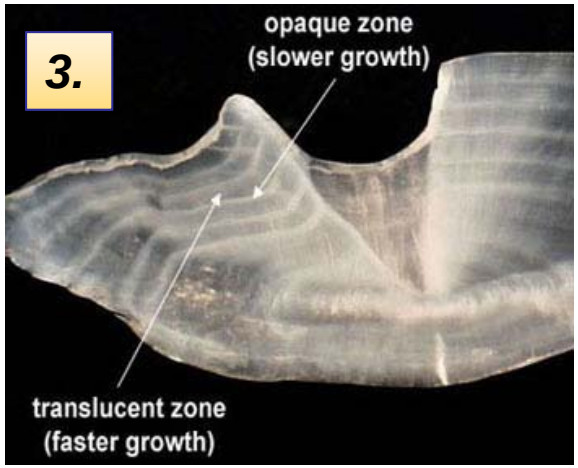
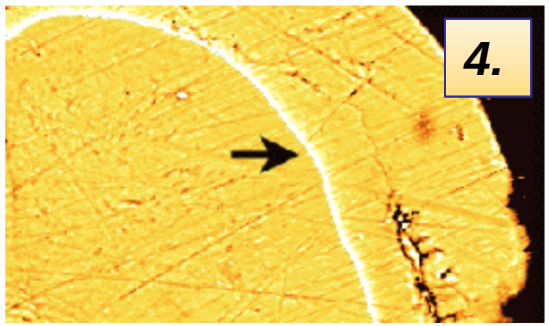


Image acquired from http://http://keywordsuggest.org

Strontium

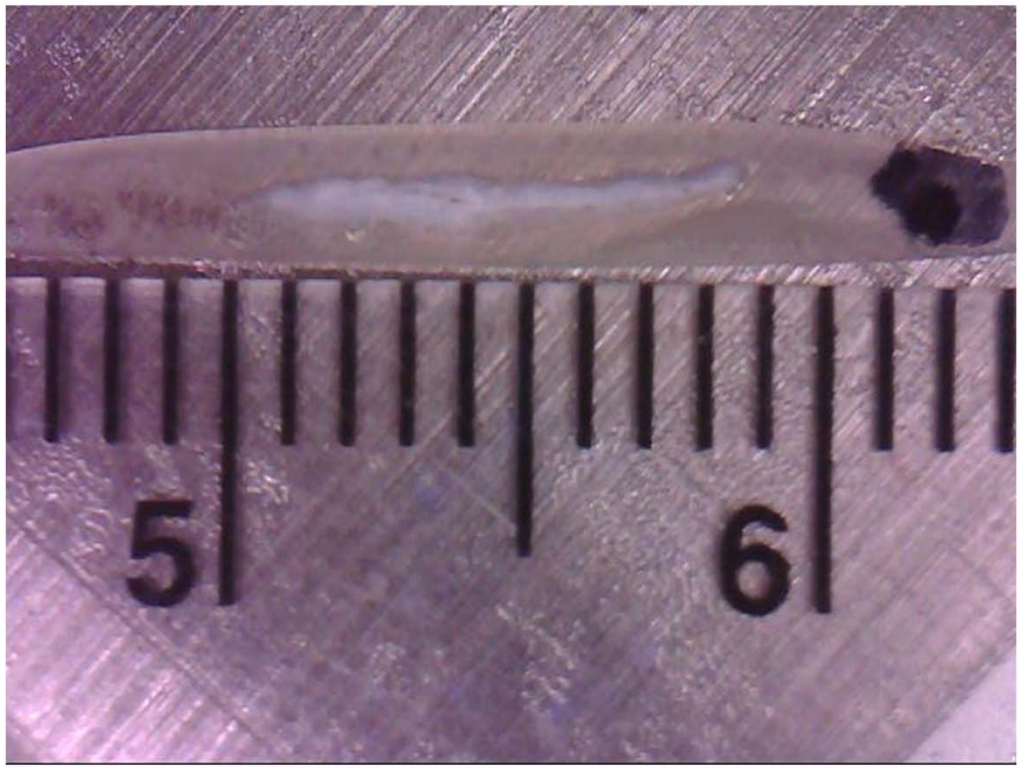


Salmonids can be successfully mass-marked using strontium chloride at any life history stage. Thermal marks, in contrast, can only be applied during a 2 to 4 week period after the eyes form in the embryos. One drawback of the strontium mark, however, is that they cannot be viewed using a traditional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

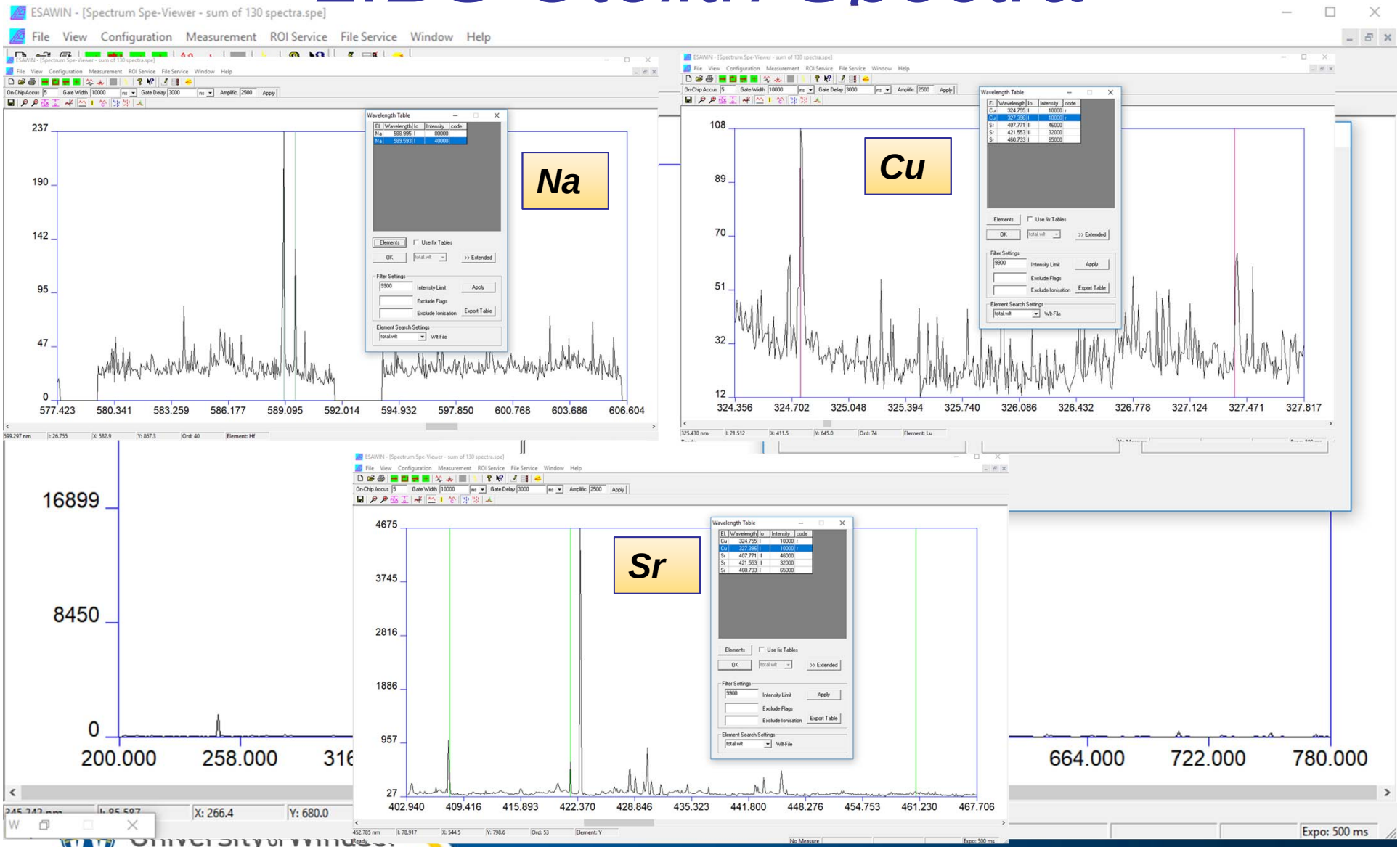
Can we monitor fresh/salt water migration via the elemental concentration?

Otolith Samples

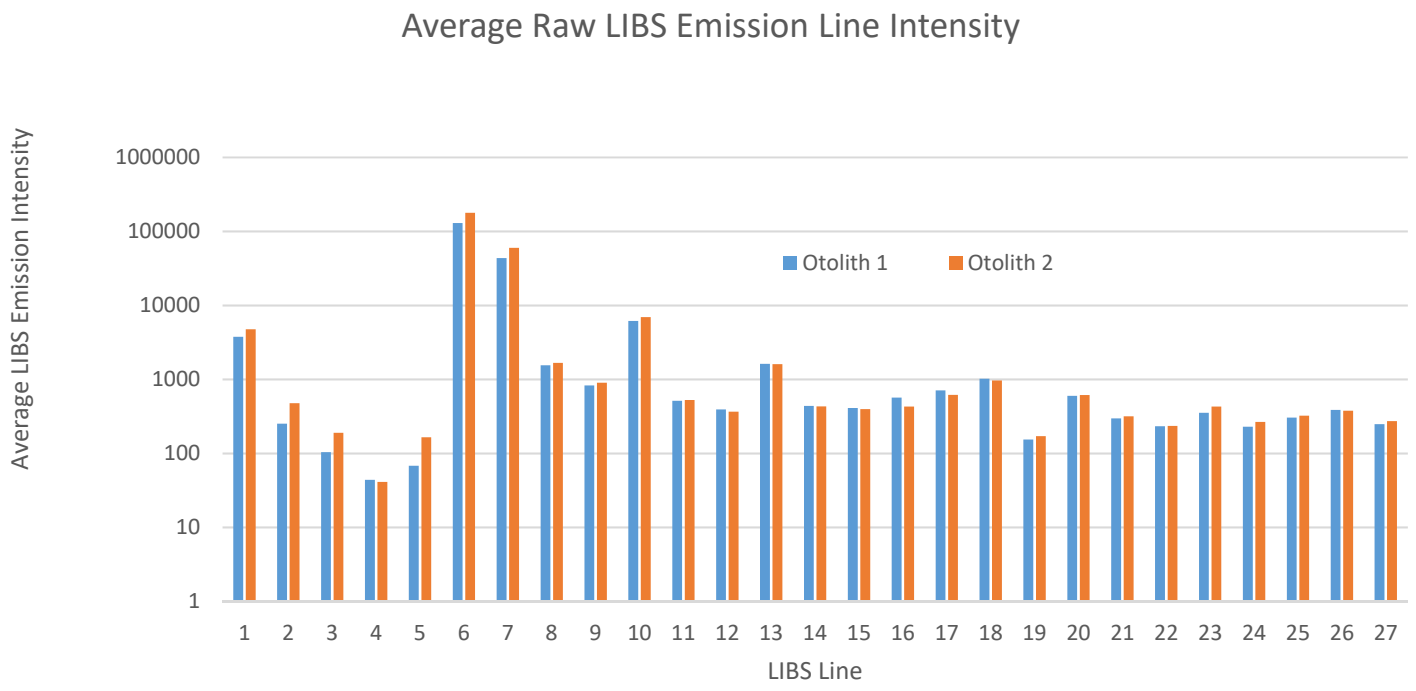
Mounted in a paraffin wax and cross-sectioned



LIBS Otolith Spectra



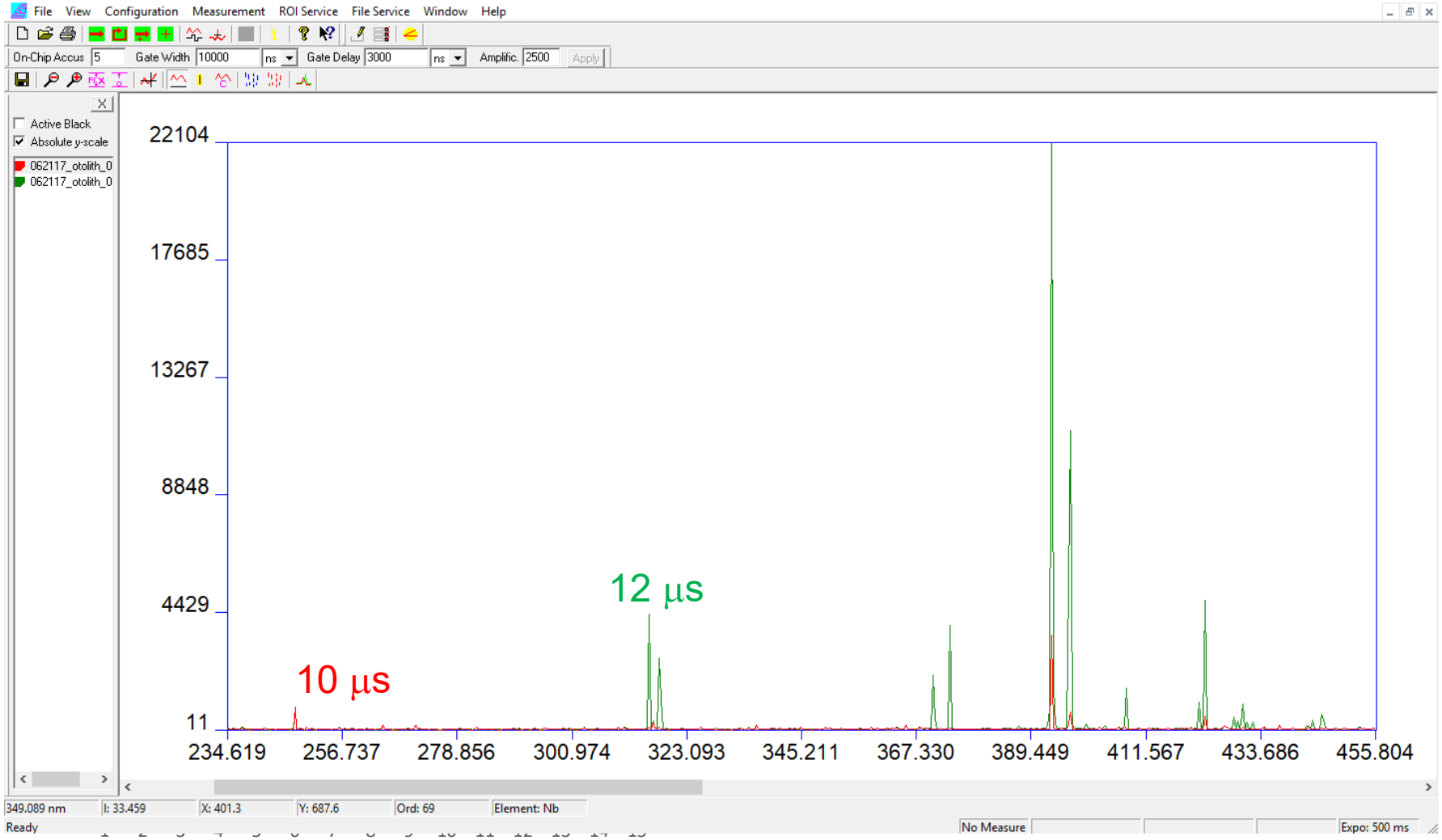
LIBS Spectra, Two Otoliths



1	C 247.856
2	Mg 279.55
3	Mg 280.27
4	Ca 300.922
5	Cu 324.755
6	Ca 393.366
7	Ca 396.847
8	Sr 407.771
9	Sr 421.553
10	Ca 422.673
11	Ca 428.937
12	Ca 429.899
13	Ca 430.253
14	Ca 430.774
15	Ca 431.865
16	Ca 442.544
17	Ca 443.497
18	Ca 445.478
19	Ca 527.028
20	Ca 558.876
21	Ca 559.447
22	Ca 559.849
23	Na 588.995
24	Na 589.593
25	Ca 616.218
26	Ca 643.907
27	Ca 646.258

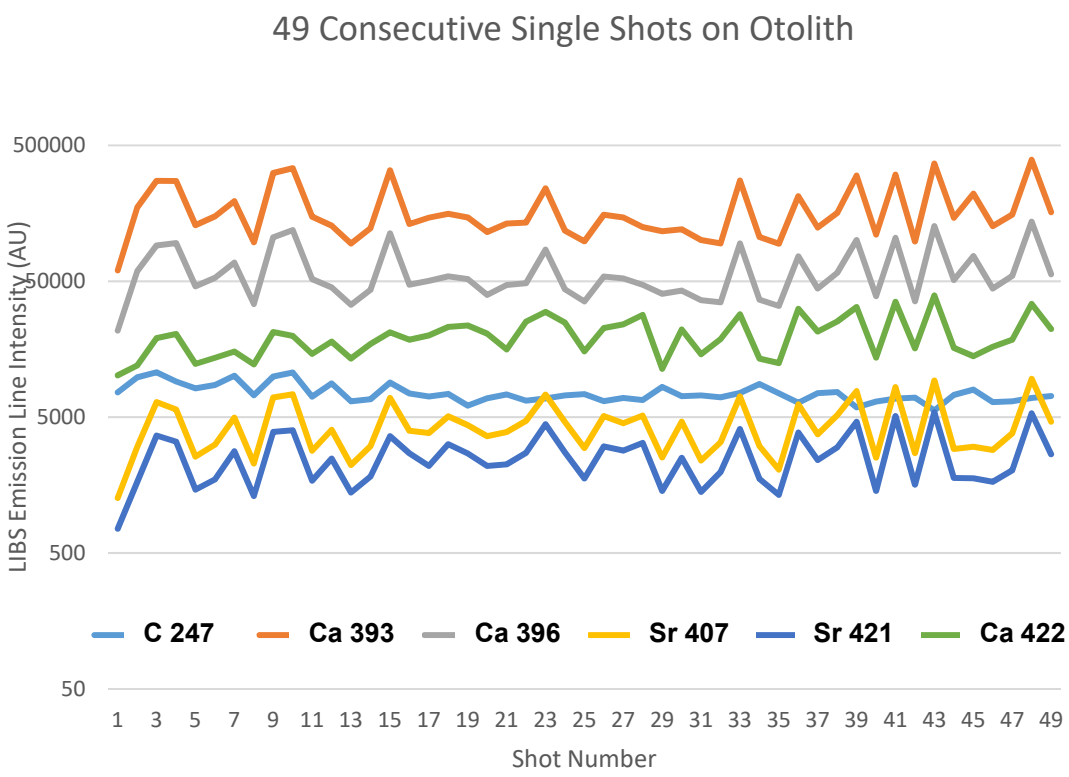
Each value an average of 65 measurements

Gate Delay Study Example



Single-Shot LIBS Not Possible

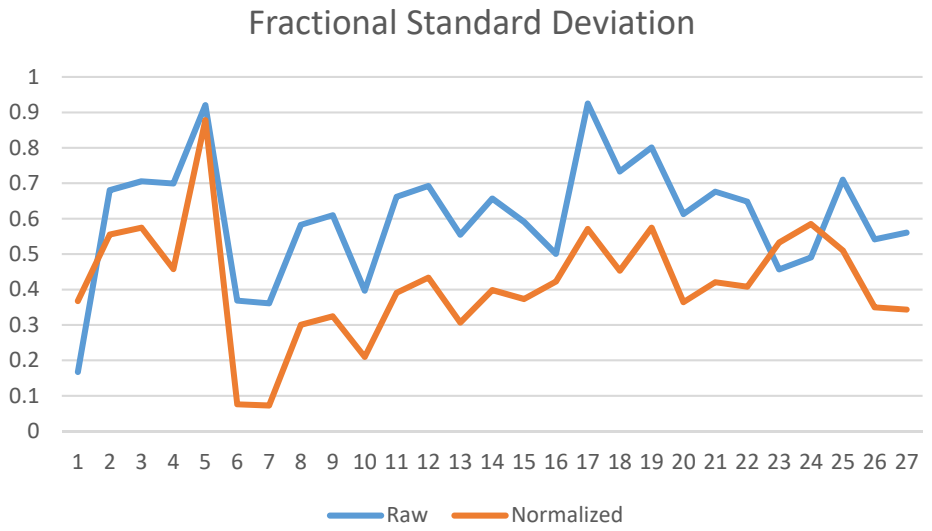
Averaging can be accomplished by “drilling” down, since we don’t have the space for lots of new locations

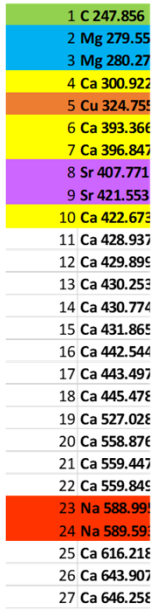


Problems With Otoliths

- Small, bony fragments can shatter easily
- Small inclusions/areas of some sort seem to yield strong increase in metal emission
- Shot-to-shot repeatability is horrible and will have to be overcome by multiple shots in one location

130 measurements of 27 lines



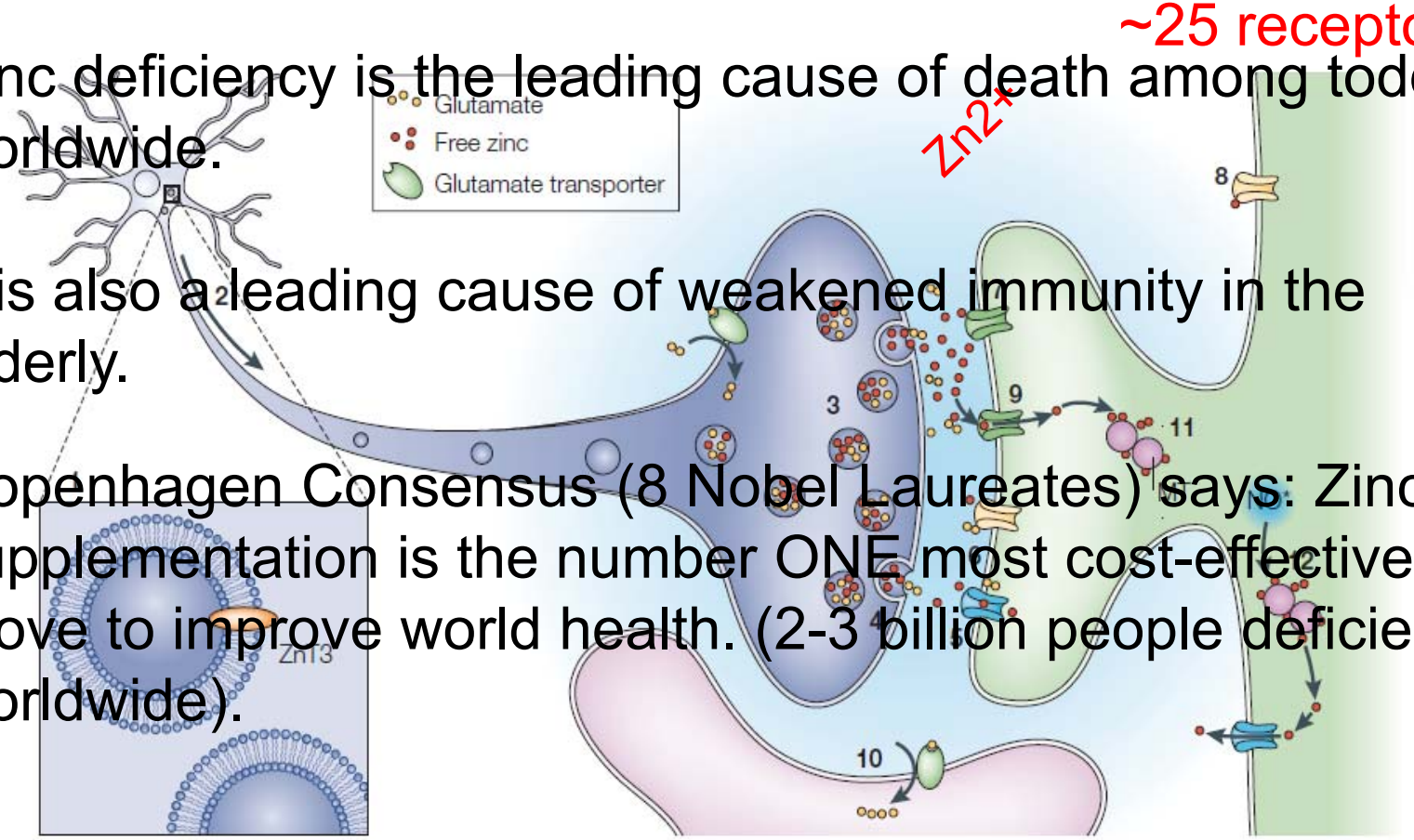


Fingernails Motivation

Zinc deficiency is the leading cause of death among toddlers worldwide.

It is also a leading cause of weakened immunity in the elderly.

Copenhagen Consensus (8 Nobel Laureates) says: Zinc supplementation is the number ONE most cost-effective move to improve world health. (2-3 billion people deficient worldwide).



Frederickson et al., *Nature Neuroscience*, 2003

How do we diagnose and monitor zinc deficiency & remediation in 2-3 billion people?

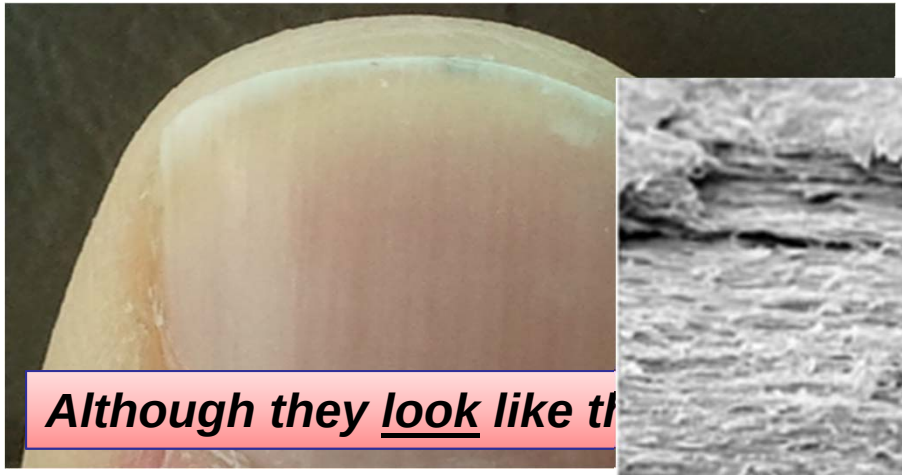
Can LIBS do this?

Why

represent t ve nc ntrati the body.

But we need a rea time biomedical assay

The Problem With Fingernails



Although they look like th

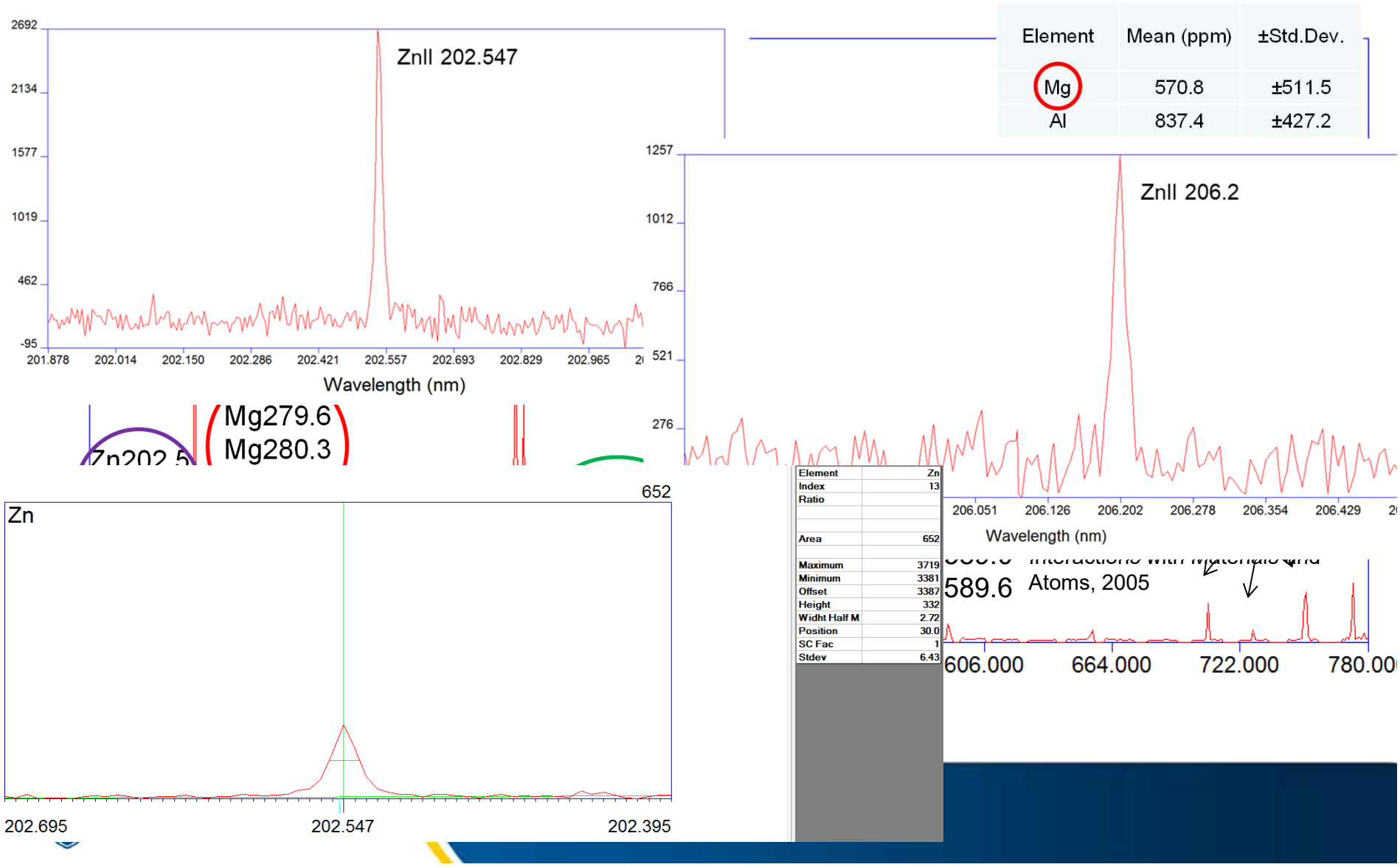
…they are actually like this!

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

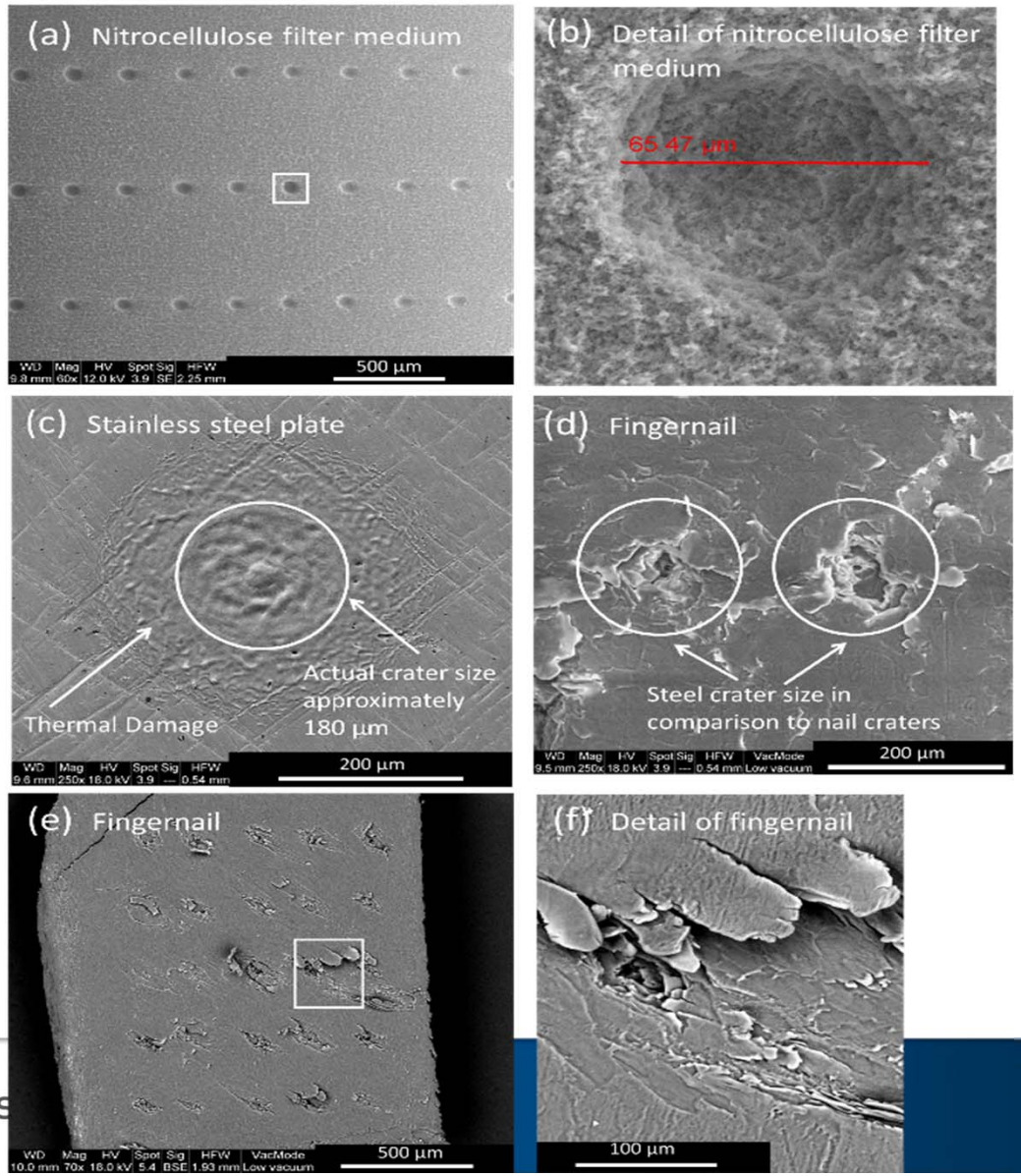
Preparation of Nails

- 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.

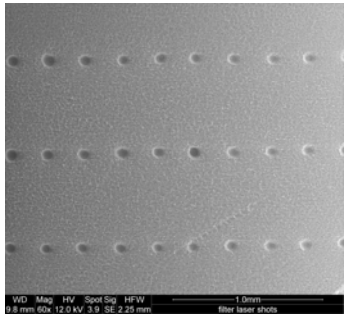
Typical Nail Components

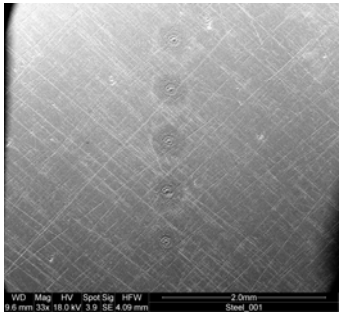


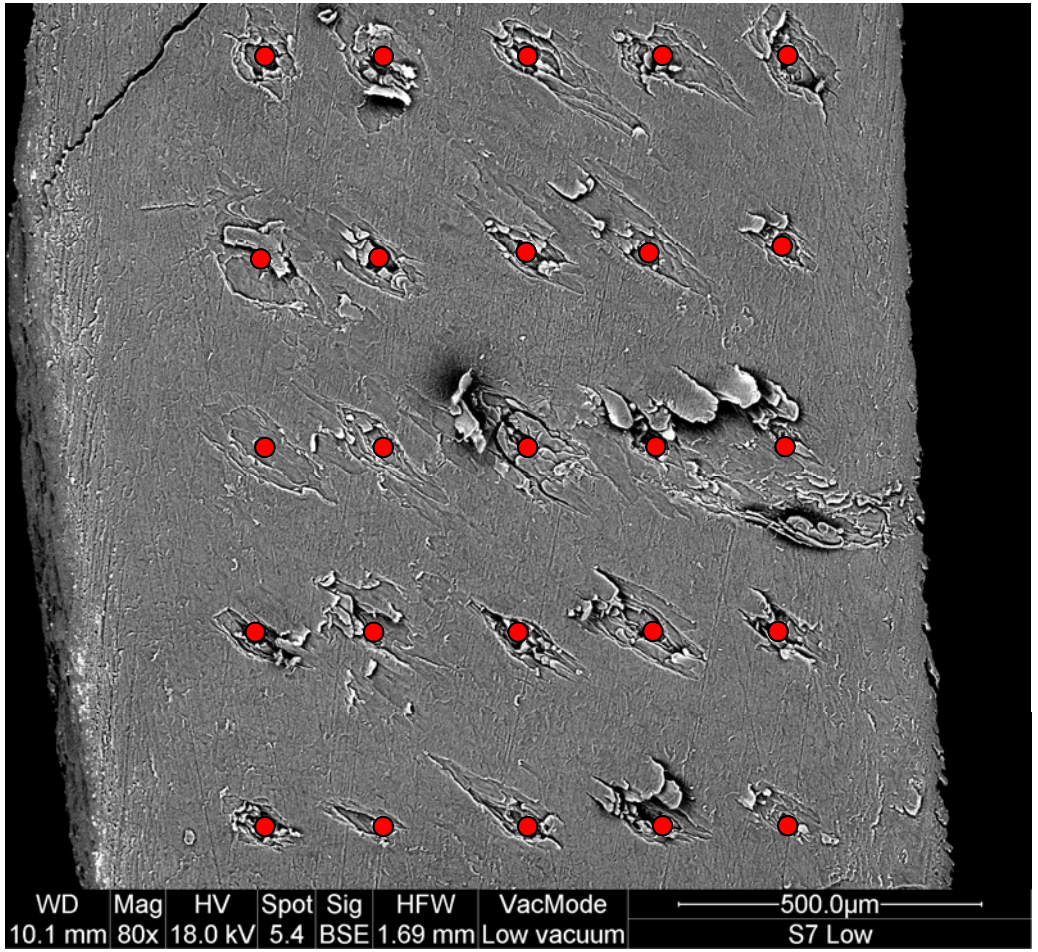
Zinc Easy to See, Harder to Quantify



25 “Identical” Shots?



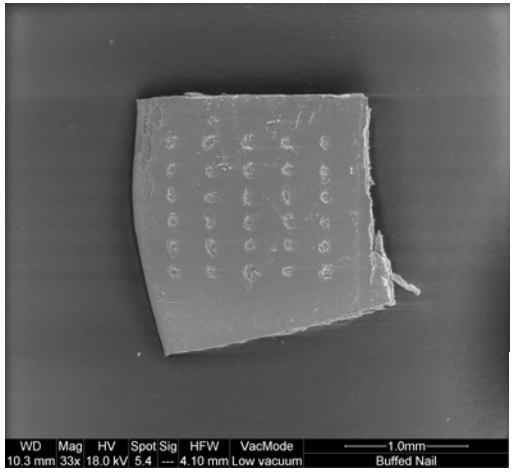




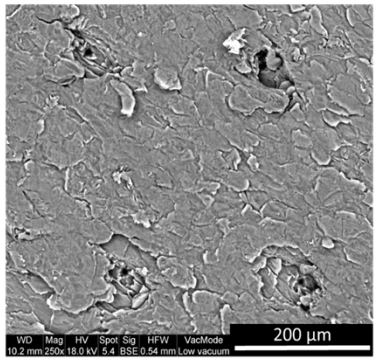
Shot-to-shot Irreproducibility

We tried:

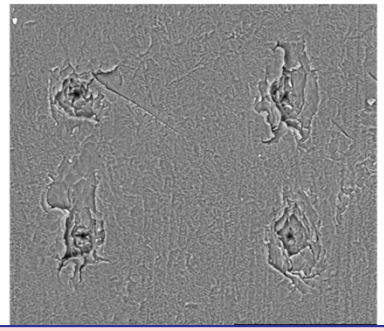
- Hydration
- De-hydration
- Surface buffing



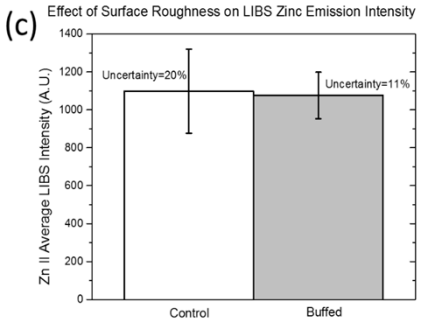
(a)



(b)

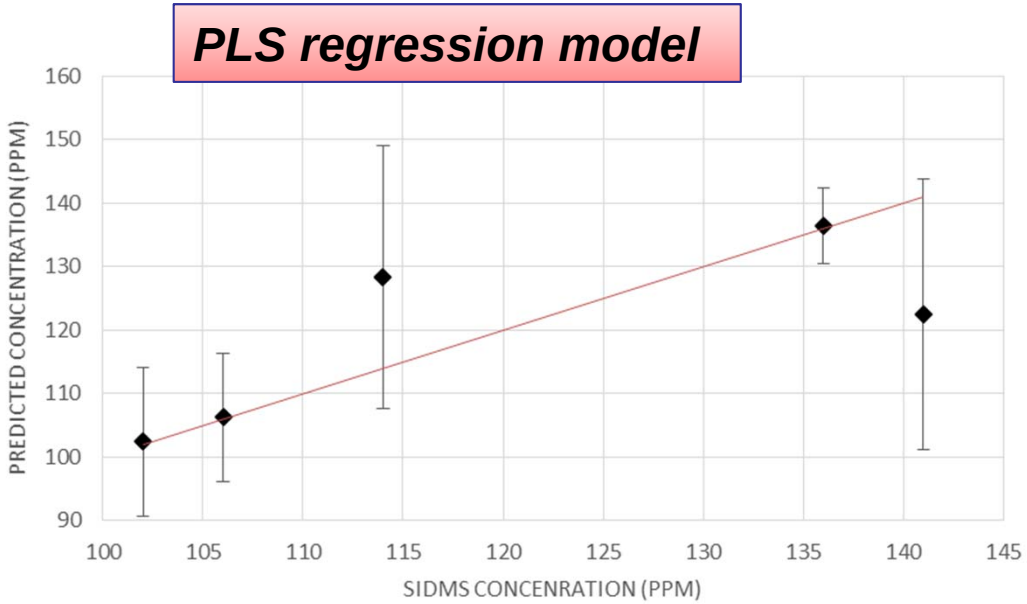


(c)



Buffing did reduce shot-to-shot variations

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.

Results

Applied Spectroscopy

Article

Applied Spectroscopy
2017, Vol. 71(4) 567–582

DOI: 10.1177/0003702816687568
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Determination of the Zinc Concentration in Human Fingernails Using Laser-Induced Breakdown Spectroscopy

Vlora A. Riberdy[1], Christopher J. Frederickson[2,3], and Steven J. Rehse[1]

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Talanta

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Quantitative determination of calcium, magnesium, and zinc in fingernails by laser-induced breakdown spectroscopy

David A. Rusak*, Ann E. Zeleniak, Jillian L. Obuhosky, Scott M. Holdren, Craig A. Noldy

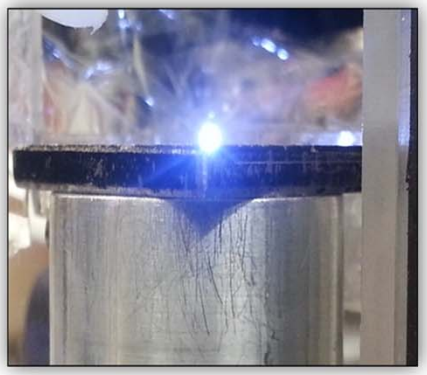
Department of Chemistry, University of Scranton, Scranton, PA 18510, USA

Bacteria

Trypticase soy agar (TSA) is used to culture bacteria.

Colonies are removed and placed in 1.5 mL of deionized water.

Sample is vortexed and 30 μL is deposited on a 0.45 μm nitrocellulose filter paper.

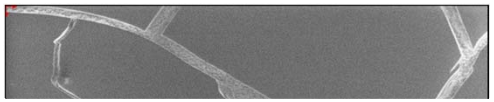


Filter paper is mounted on steel piece and ablated with laser.

Method 1

30 µL of vortexed

Colloidal suspension



Applied Spectroscopy

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Article

Applied Spectroscopy
2016, Vol. 70(3) 485–493

DOI: 10.1177/0003702815626673
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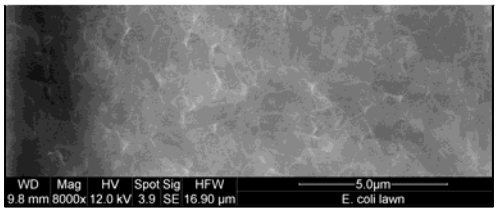
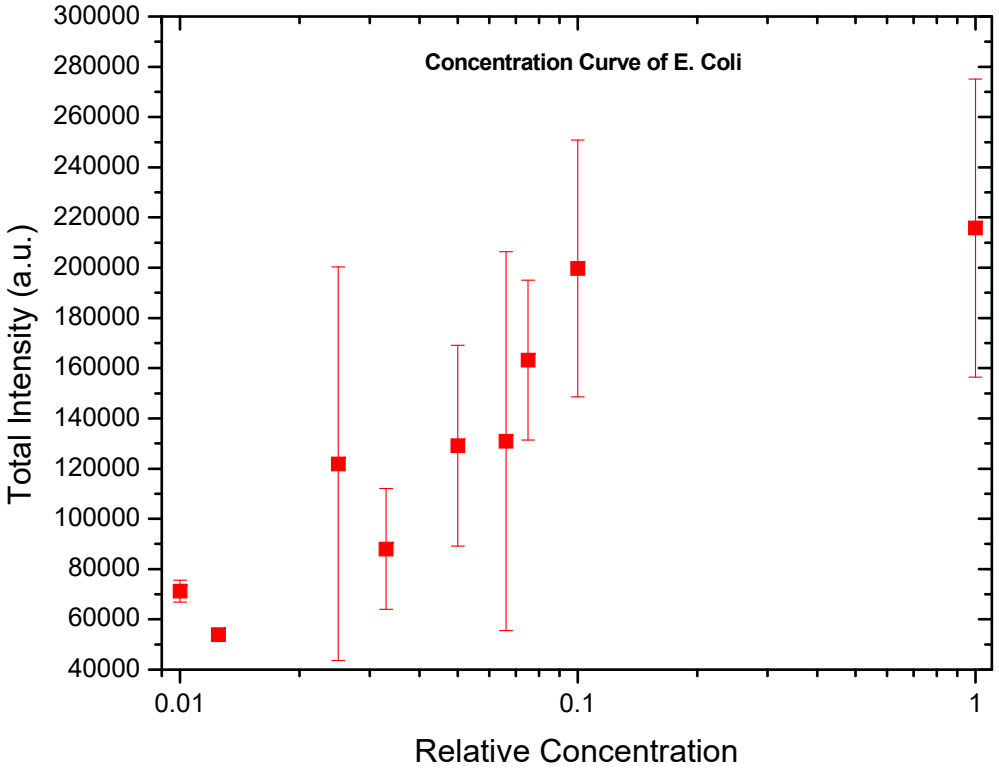
Bacterial Suspensions Deposited on Microbiological Filter Material for Rapid Laser-Induced Breakdown Spectroscopy Identification

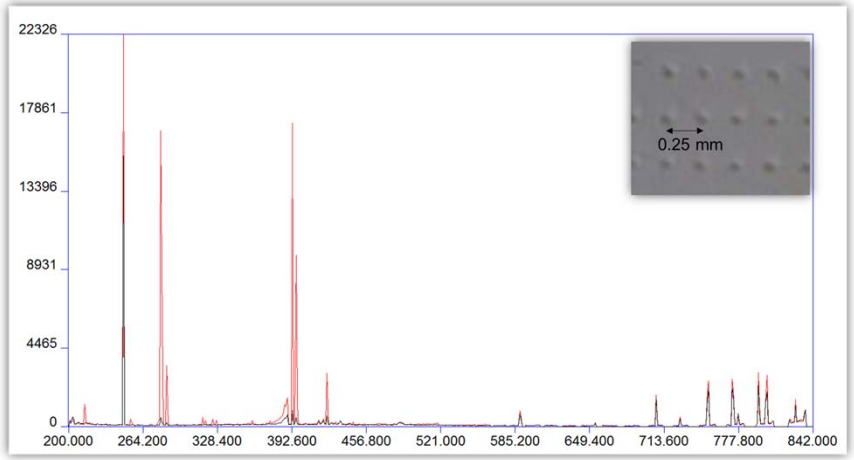
Dylan J. Malenfant, Derek J. Gillies, and Steven J. Rehse

so-nicely, but that's okay, we need a much smaller number anyway



Lowering the Cell Titer

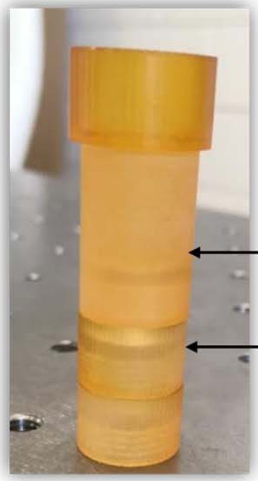




Two nominally “identical” sampling locations

Method 2

An insert for a centrifuge tube was designed by a previous student as a tool for depositing bacterial suspensions on filter media.



Bacterial Mounting and Concentration Techniques to Translate Laser-Induced Breakdown Spectroscopy into a Clinical Setting

University of Windsor

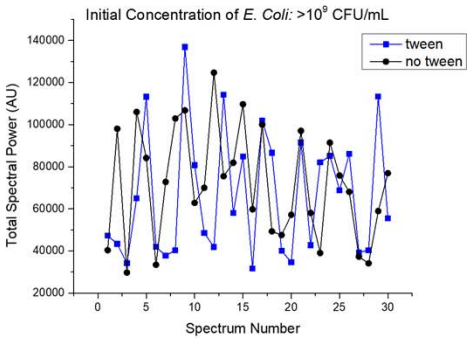
Alexandra Paulick, Naila Rahman, and Steven J. Rehse

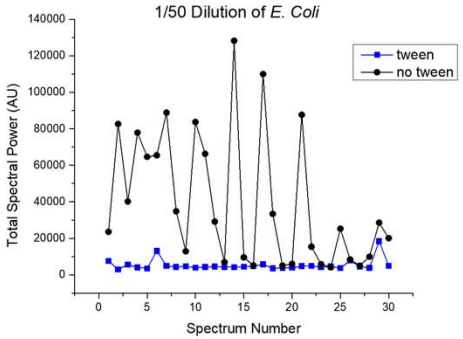
Department of Physics, University of Windsor, Windsor, ON, CAN

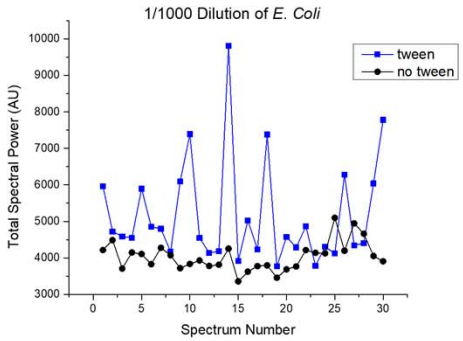
NSERC CRSNG

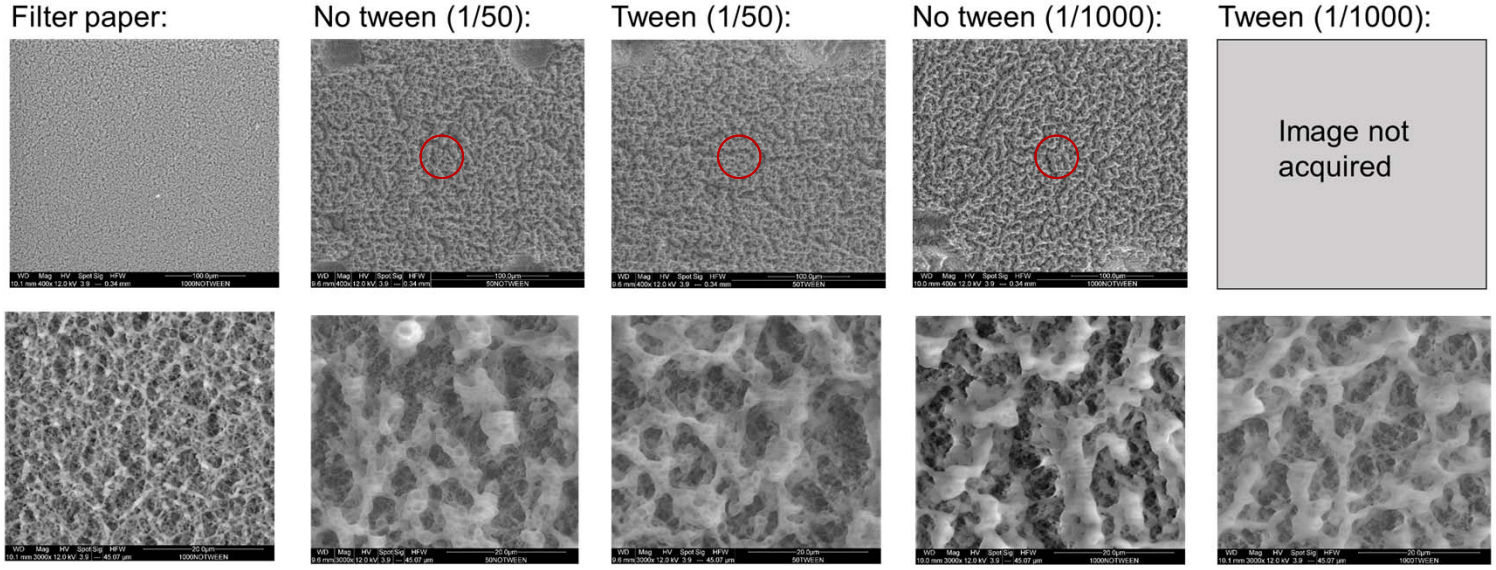
FACSS PRESENTS SCIX2017

Treatment with Tween 20





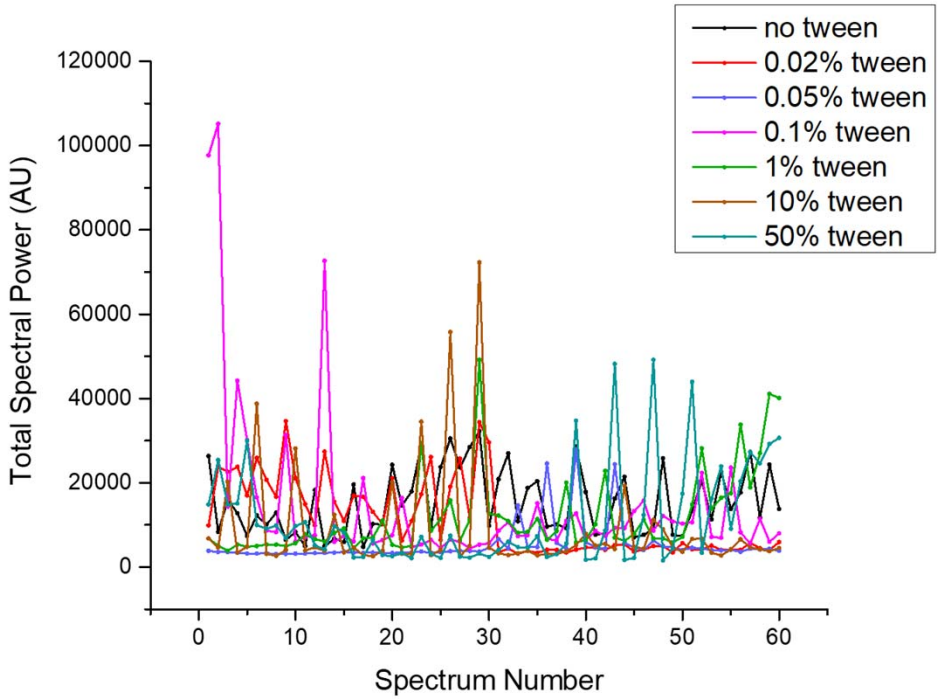




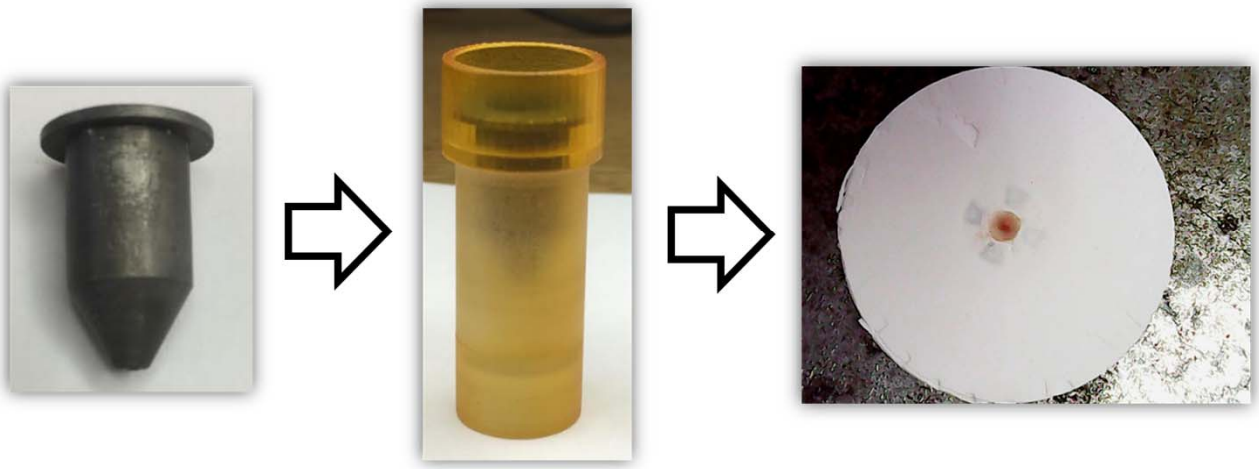
- Dilutions treated with 0.1% Tween 20
- 30 spectra acquired across filter (each an average of 3 single-shot spectra)

Treatment with Tween 20

- Various concentrations of Tween were added to *E. coli* suspensions of the same concentration.
- Each suspension was deposited on a different filter medium and 60 LIBS spectra were acquired.
- There does <u>not</u> seem to be a specific concentration of Tween that yields a relatively constant LIBS bacterial signal around the average LIBS signal of the clumped bacteria.



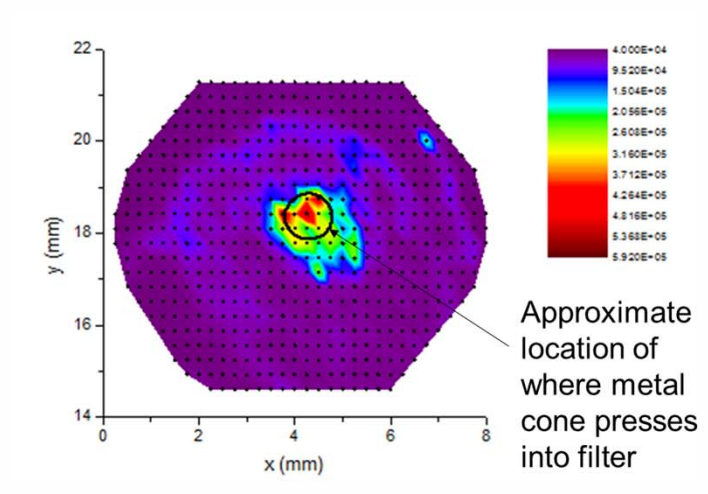
Method 3



Bacteria is forced onto a smaller area of the filter paper, increasing the number of cells ablated per laser shot.

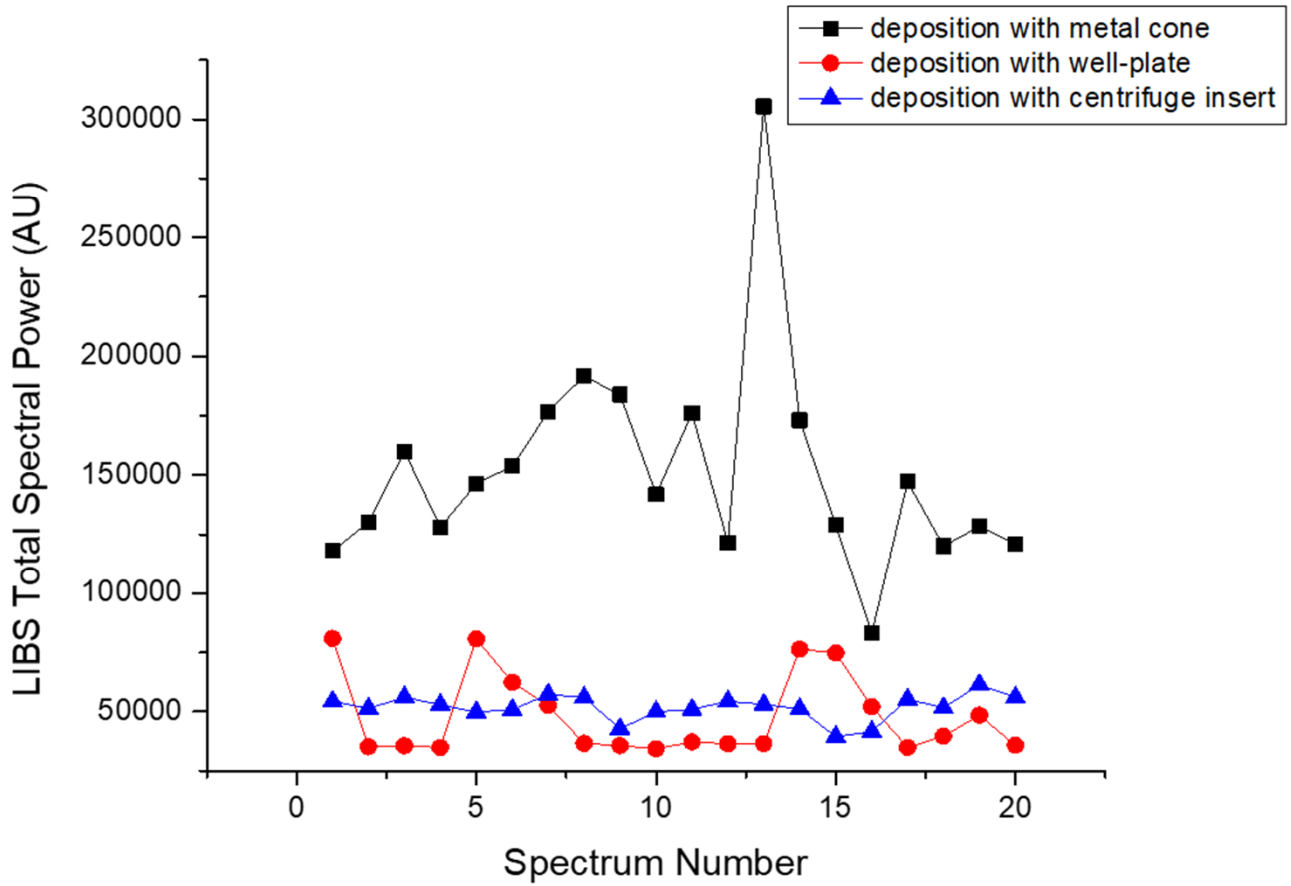
The metal cone was used to deposit an *E. coli* suspension on a filter paper and 570 LIBS spectra were acquired across it to obtain an intensity map of the bacterial deposition on the filter.

Each point on the map corresponds to a 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.



Method 3

The same *E. coli* suspension was deposited on filter papers using different deposition methods, and 20 spectra were acquired across each filter.



Method 4

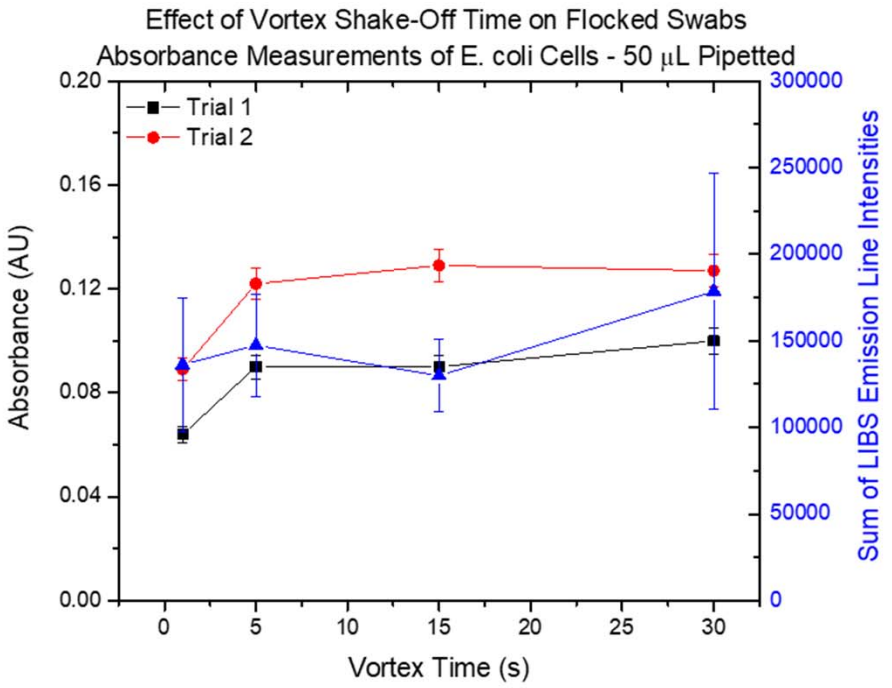
Standard swab test

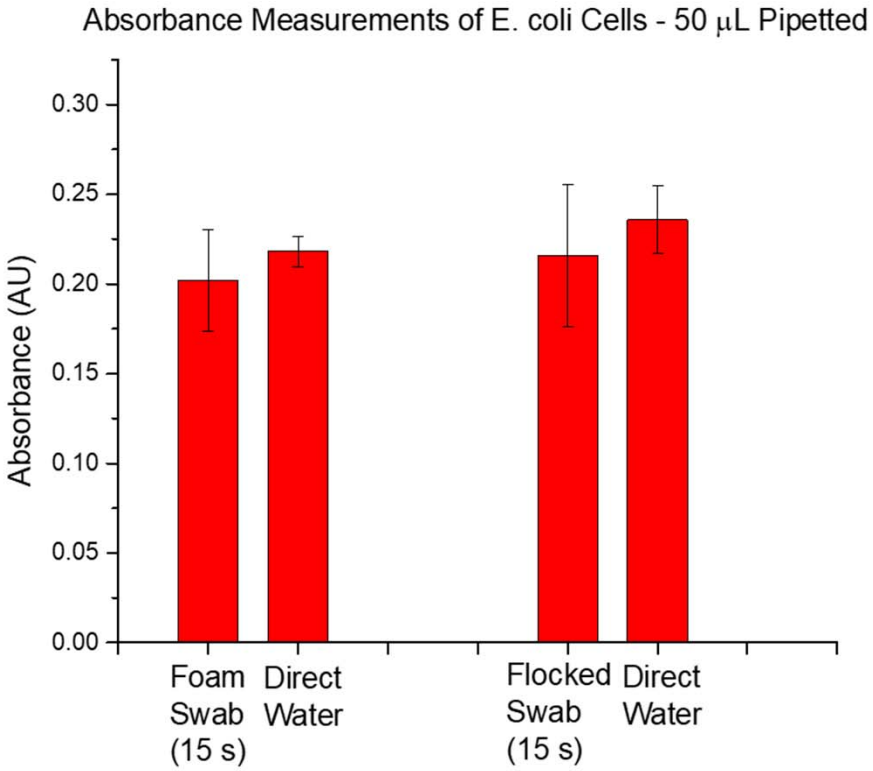
Method 4

Cannot shoot right on the swab

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

Swab Vortex Shake-Off





Conclusions

- Although the otoliths, nails, and bacterial films are all composed of one material, their physical non-uniformity results in enhanced (or greatly enhanced) shot-to-shot variation.
- Different sampling strategies are needed to overcome this.
- Specific new sample-preparation steps are needed to overcome this.
- Perhaps “outlier exclusion” should be utilized?

Funding and Acknowledgements

We gratefully acknowledge funding for this project provided by:

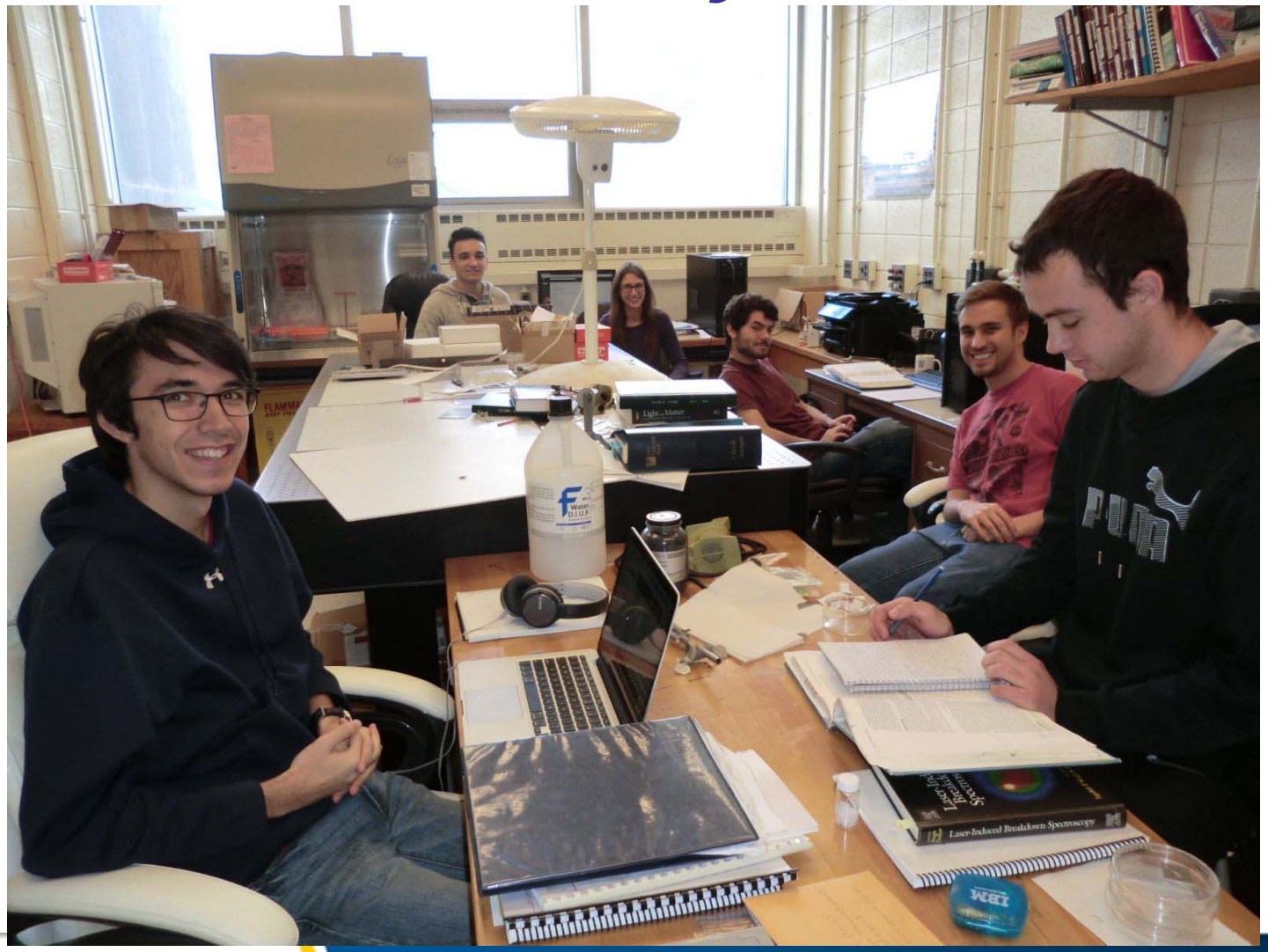
- A *Natural Sciences and Engineering Research Council of Canada* Discovery grant and a Research Tools and Instruments grant

- 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

Thank you!



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New students (grad or undergrad) always welcome!!!