Toward the Use of LIBS for the Clinical Diagnosis of Disease-Causing Pathogens

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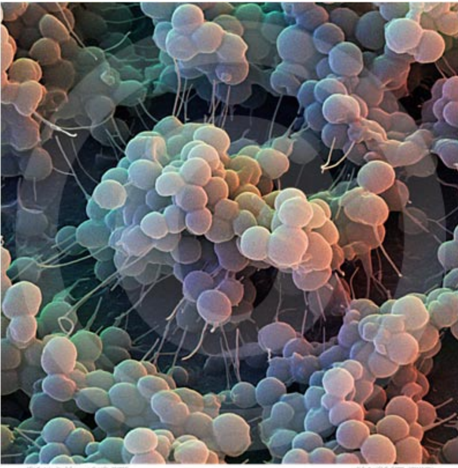
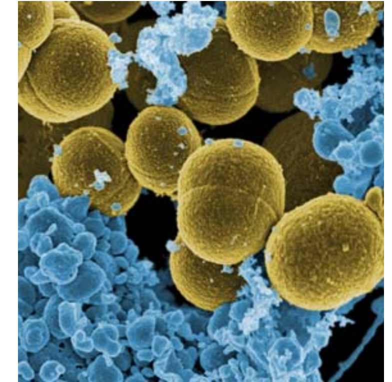


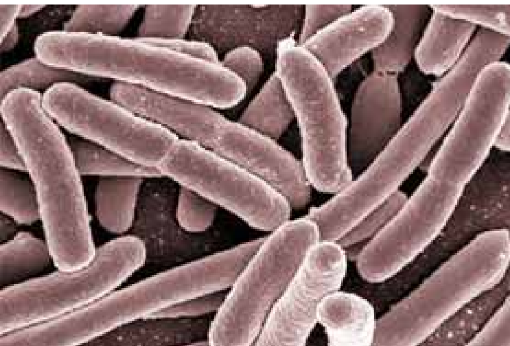
Staph. epidermidis

Staph. aureus

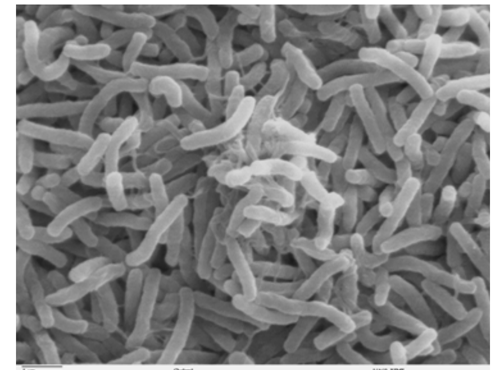


there is an urgent need right now in the military, civilian (hospital, food processing, environmental), and first responder communities for a "…rapid point-of-care (multiplex?) diagnostic for disease-causing pathogens."

E. coli



V. cholerae



How do we identify bacteria?

4 ways

- genetic
- serological (antigenic)
- microbiological
- compositional
  - LIBS
  - Raman
  - MALDI-TOF-MS

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Bruker Daltonics

MALDI Biotyper: The next generation microbial identification system for the 21st century

The MALDI Biotyper enables an unbiased identification of microorganisms. It can be applied to gram-positive and gram-negative bacteria, yeast and multicellular fungi without any presumptions or pretesting. Starting from culture plates identification results can be generated in a couple of minutes. The MALDI Biotyper covers applications from clinical microbiology, food and feed safety and analysis, as well as industrial quality control.

The MALDI Biotyper for identification of microorganisms is a system that meets all the demands defined for a revolutionary new approach - based on advanced, yet well acknowledged technology: mass spectrometry.

Bruker offers the next generation for identifying microorganisms in your lab:

- Easy sample preparation
- Fast
- Robust
- Reliable mass spectrometric instrumentation
- Easy to use software (non MS-expert approved)

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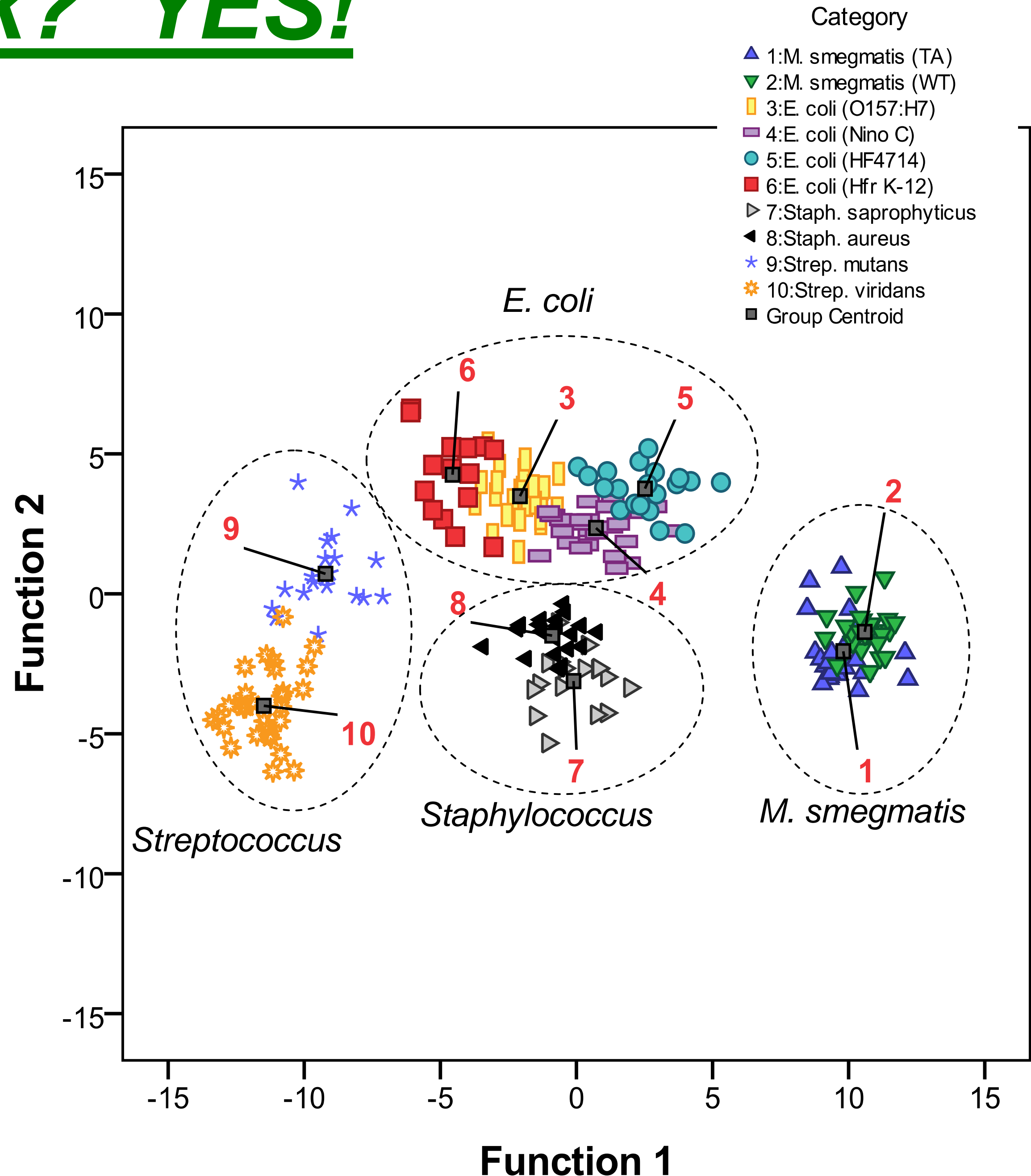
things that make a LIBS-based technology unique*

- lack of complicated sample preparation
- no expertise required
- no genetic or antigenic precursors (consumables) necessary
- speed / portability / durability (ruggedness)
  - "rapid point-of-care diagnostic…"
- same technology / hardware useful for explosives, chemical, other threats (CBRNE capable)
- capability of sensor fusion

Does it work? YES!

- Intensity of lines, ratios of intensities used in a statistical multi-variate analysis
- Discriminant function analysis (DFA)
  - principal component analysis (PCA)
  - partial least squares – discriminant analysis (PLS-DA)
  - linear discriminant analysis (LDA)



We Must Proceed, and Faster…

LIBS research must proceed along two equally important avenues:

- fundamental research to explore the microbiological diversity that can occur in specimens
- specimen preparation and handling protocols and techniques to isolate pathogens from contaminants of biological origin

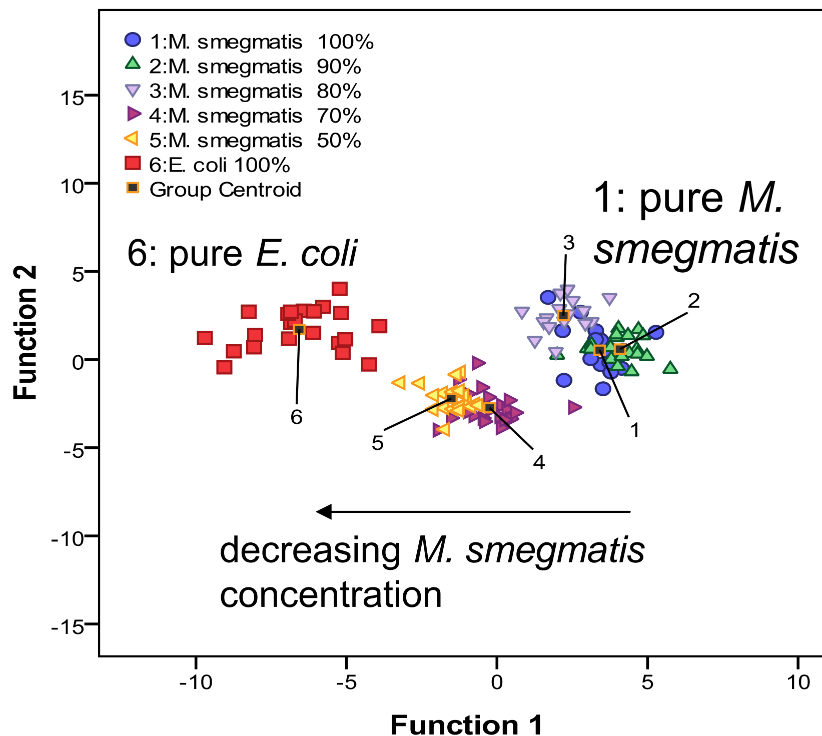
NOTE: we do NOT need to fingerprint hundreds and hundreds of "new" bacteria

The Wayne State Team is making progress on both fronts…

Biological Diversity

“Mixed” Samples

Category	# of Spectra	Classification Results		
		M. smegmatis	*E. coli*	*S. viridans*
100% *M. smegmatis*, 0% *E. coli*	21	100%	0%	0%
90% *M. smegmatis*, 10% *E. coli*	20	100%	0%	0%
80% *M. smegmatis*, 20% *E. coli*	16	100%	0%	0%
70% *M. smegmatis*, 40% *E. coli*	21	76%	24%	0%
50% *M. smegmatis*, 50% *E. coli*	19	47%	53%	0%
0% *M. smegmatis*, 100% *E. coli*	25	0%	100%	0%



- Mixtures of known mixing fraction were prepared from suspensions *M. smegmatis* and *E. coli* C.
- Six separate mixtures were prepared with a ratio *M. smegmatis* to *E. coli* C given by M_{1-x}:C_x with x = 0.0, 0.1, 0.2, 0.3, 0.5, 1.0.
- Multiple 1.5 mL tubes of these mixtures were prepared, thoroughly agitated via vortex mixing, then centrifuged for 3 minutes at 5000 rev/min.

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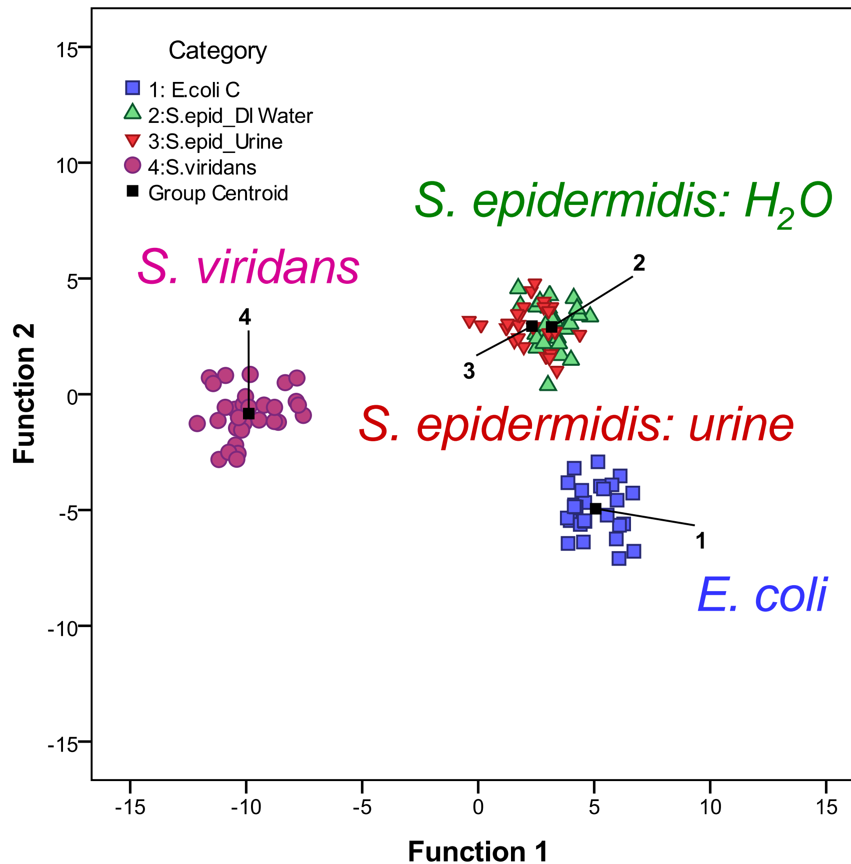
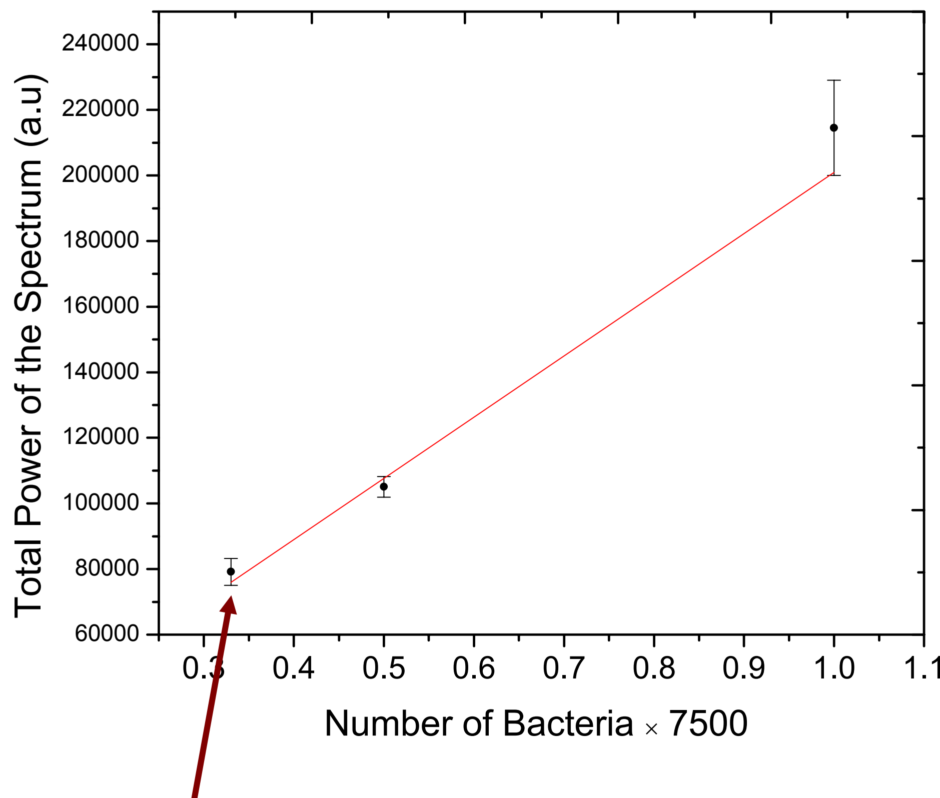
"Dirty" samples



- Samples of *Staph. epidermidis* were prepared in DI water and sterile urine.
- Samples were collected and tested via LIBS with NO WASHING.
- LIBS spectral fingerprint from urine-exposed bacteria were identical to water-exposed bacteria.
- EMMA correctly classified 100% of the urine-exposed bacteria as being consistent with *S. epidermidis*

LIBS intensity linearly dependent on number of bacteria



5 laser sampling locations

~500 bacteria per locations

- Samples of *E. coli* with different titer tested on agar.
- Each data point is the average of 5 sampling locations.
- As expected, spectra demonstrate a linear dependence with cell number.
- All spectra were 100% correctly identified (specificity not dependent on number of cells).
- Suggests an antibiotic resistance test?

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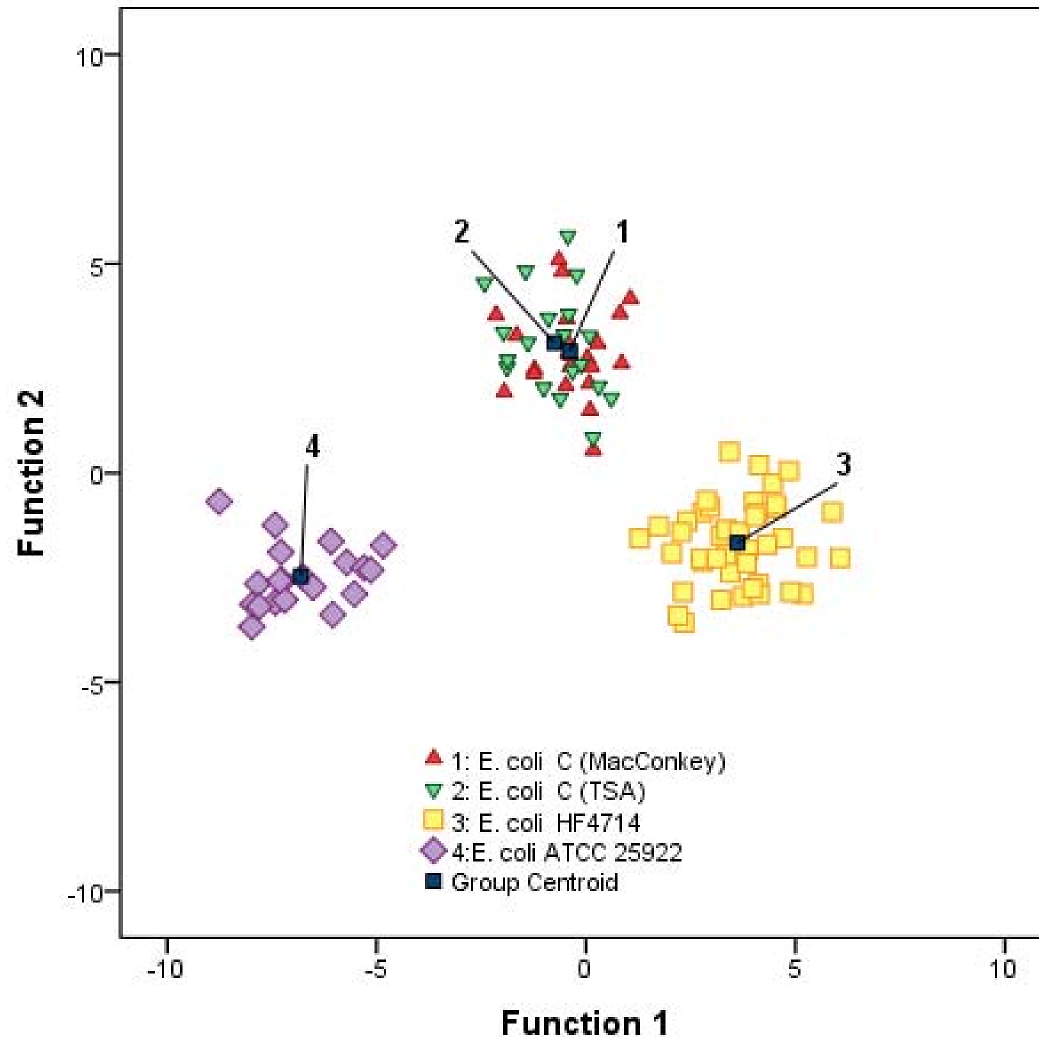
LIBS specificity not dependent on nutrition environment



- Three strain of non-pathogenic *E. coli*
- One strain grown in multiple media (1&2)
- All strains 100% accurately identified

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Strain discrimination confirmed

The Use of Laser-Induced Breakdown Spectroscopy for Distinguishing Between Bacterial Pathogen Species and Strains

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Applied Research Associates, Inc., 4300 San Mateo Blvd NE Suite A-220, Albuquerque, New Mexico 87110 (R.A.M., D.A.C.); and Department of Biology, New Mexico State University, P.O. Box 30001, Las Cruces, New Mexico, 88003-8001 (J.M.D., J.G.)

APPLIED SPECTROSCOPY Volume 64, Number 7, 2010

- 100% accuracy exhibited in blind trials of 4 MRSA strains and one *E. coli* strain
- lyophilized ("freeze-dried") specimens used

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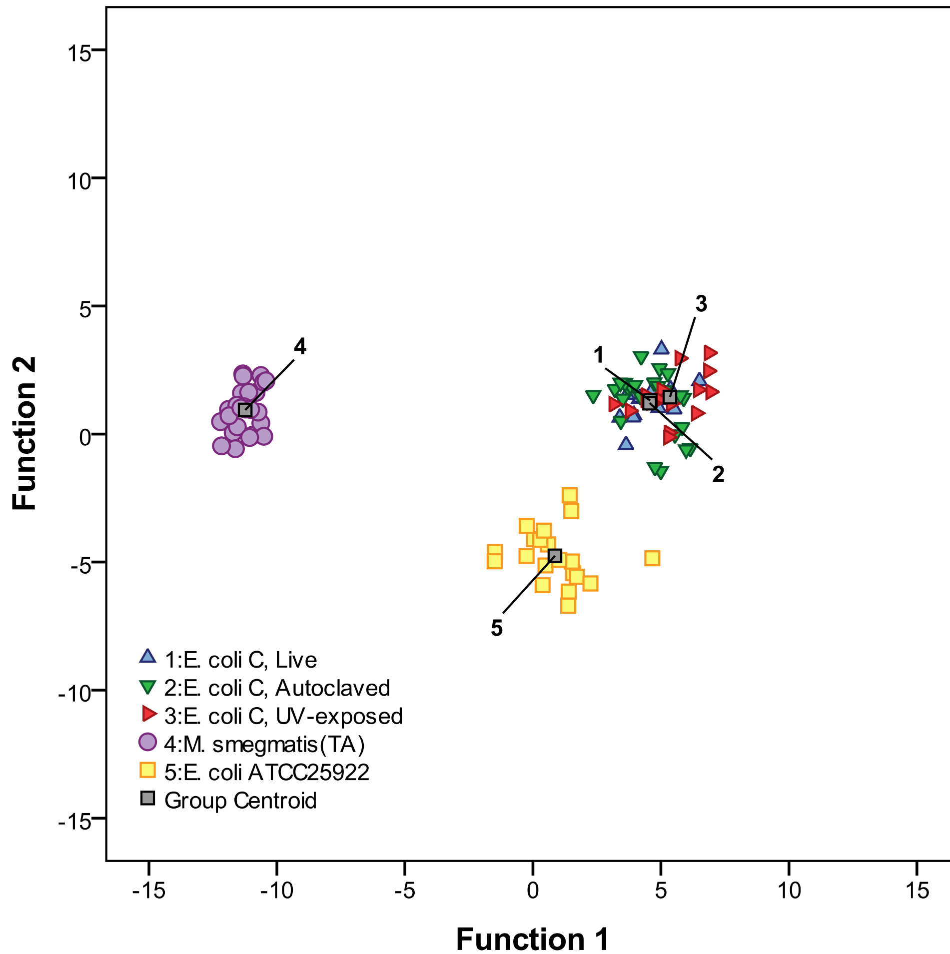
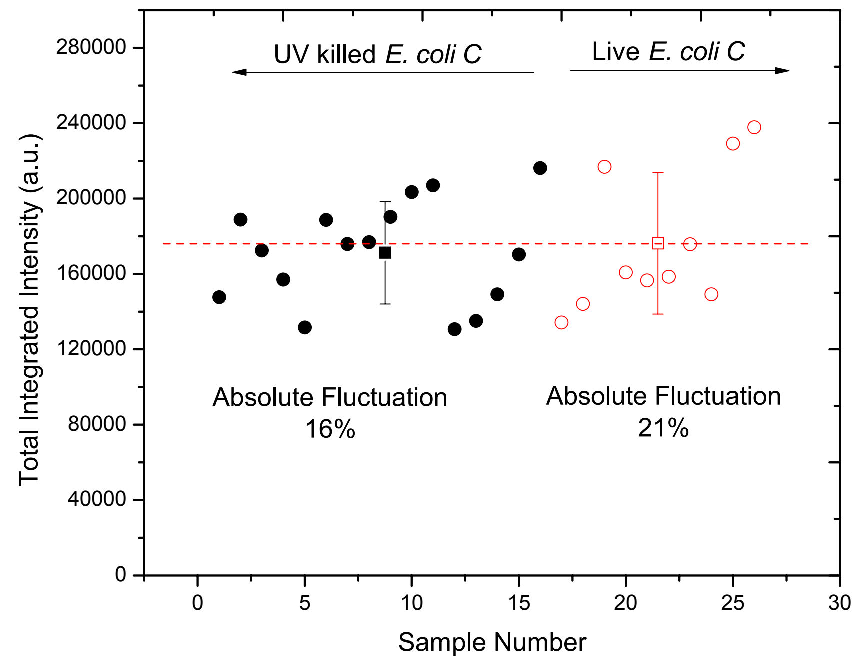
LIBS specificity and sensitivity not dependent on bio-activity of the bacteria





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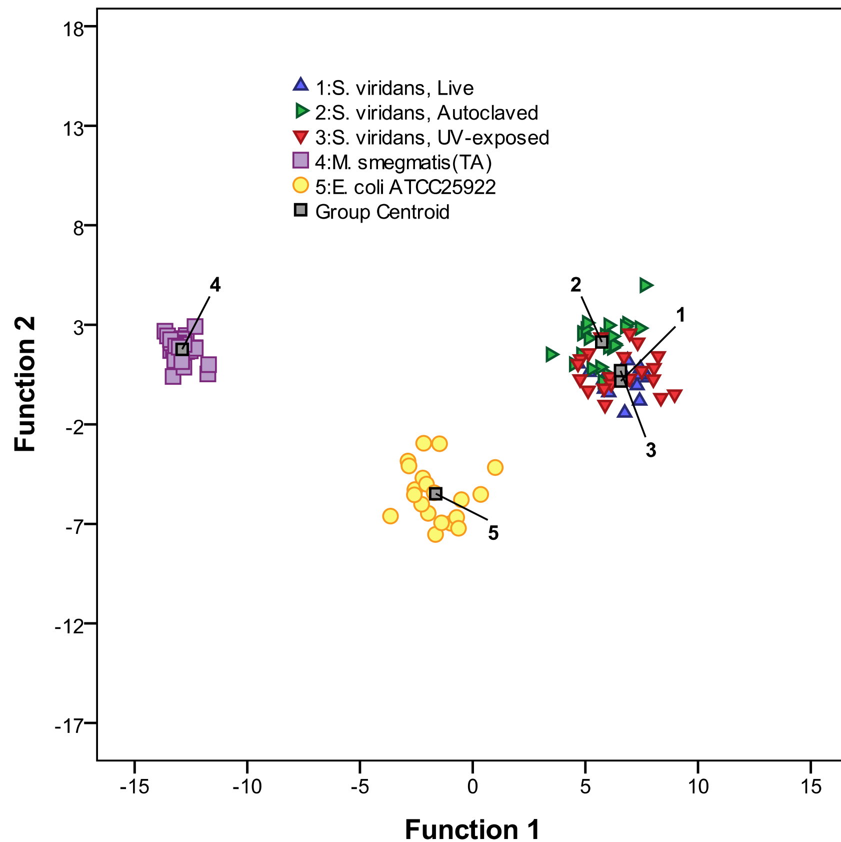

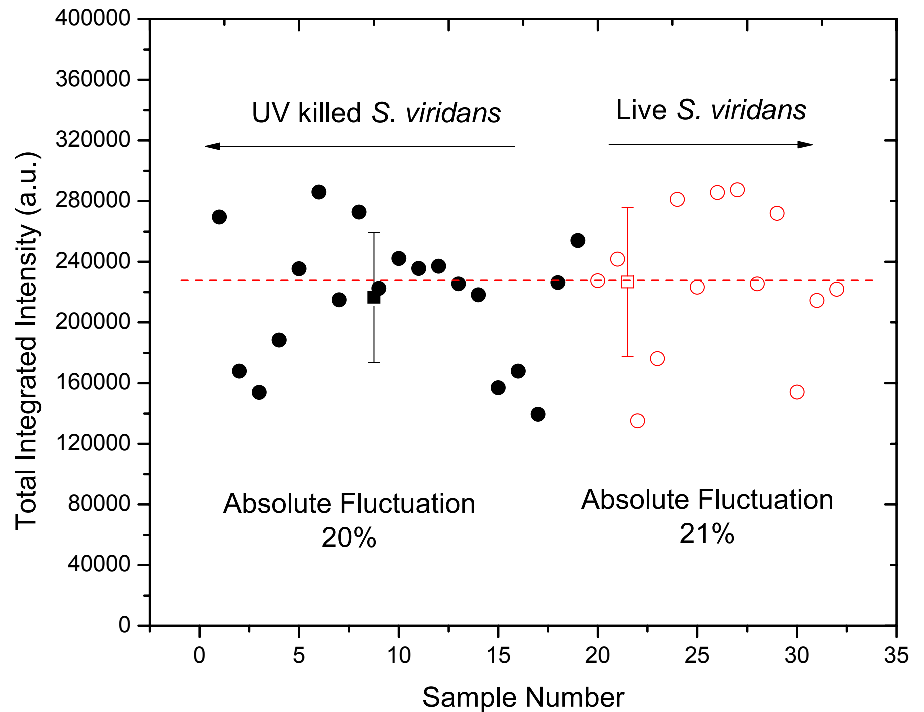

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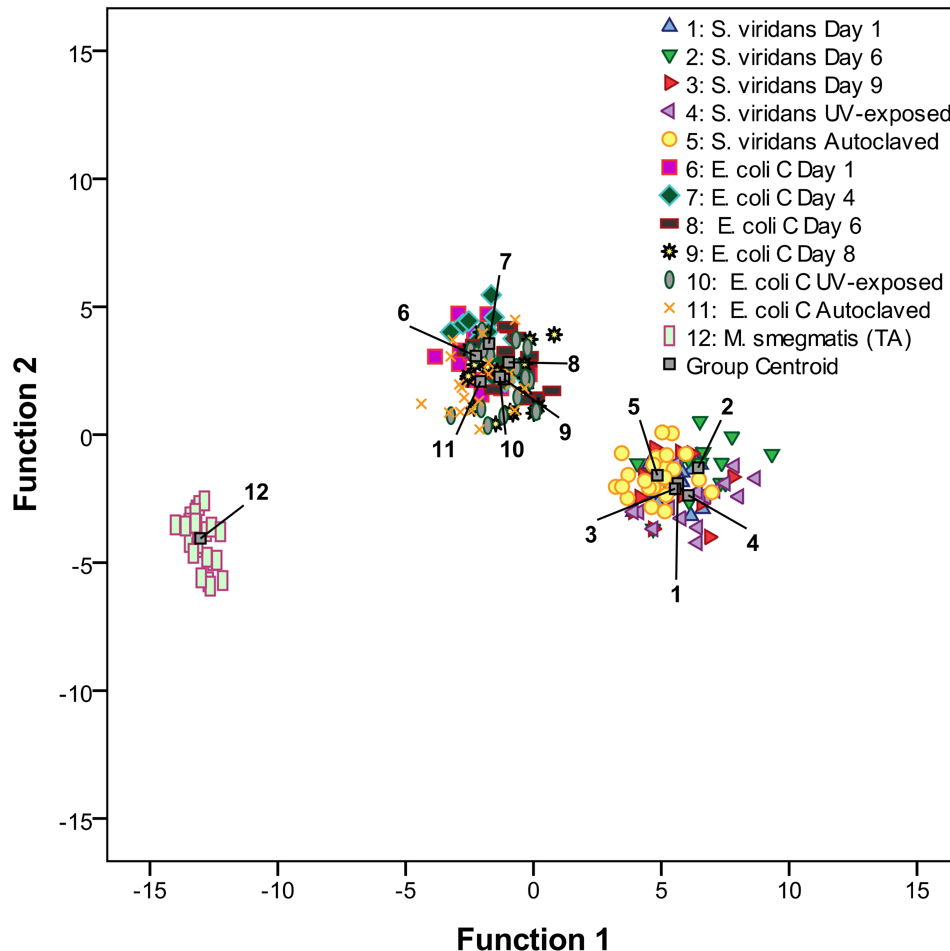
LIBS specificity and sensitivity not dependent on bio-activity of the bacteria



- Two species of bacteria tested
- All specimens prepared separately and left to sit on a nutrient-free medium for up to 9 days at room temperature
- This graph also includes the UV-irradiated and the autoclaved specimens
- All species 100% accurately identified

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The Wayne State Team is making progress on both fronts…

Specimen handling

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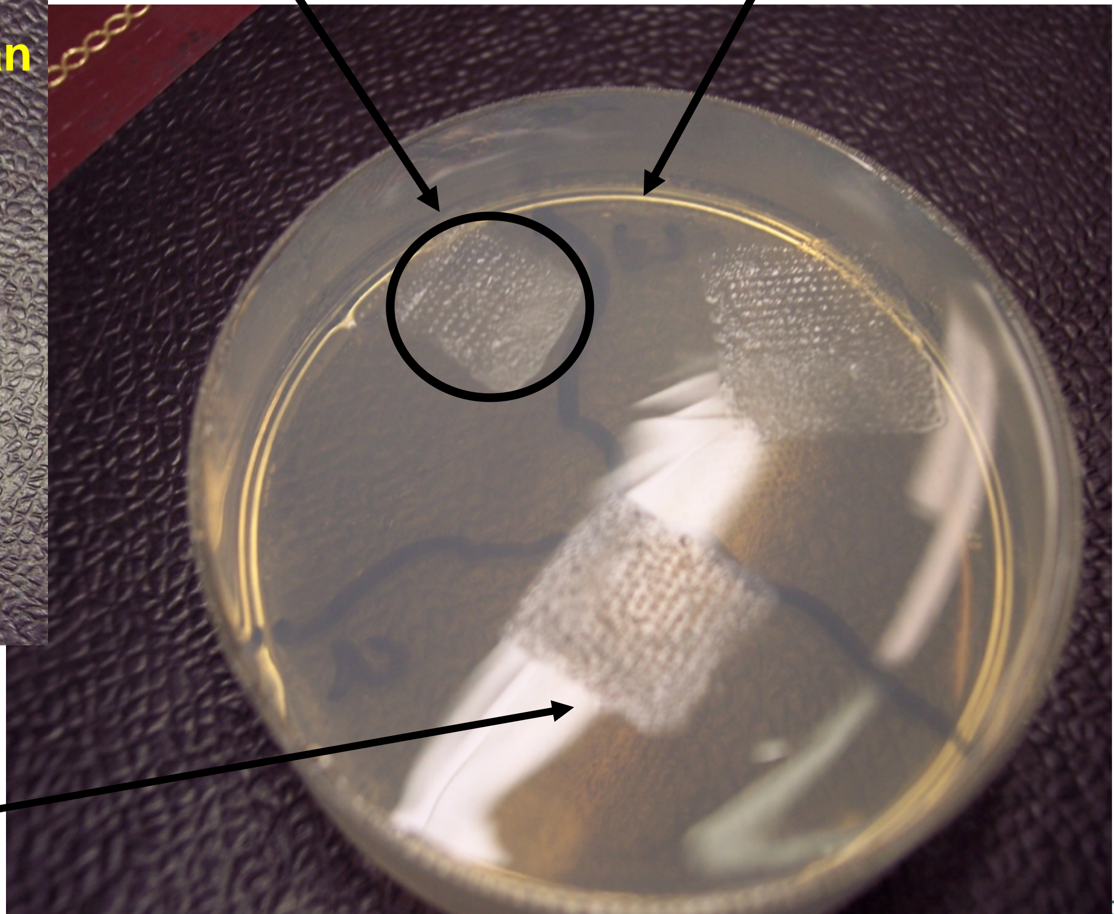
how we did it…

10 microliter of bacteria pellet

bacto-agar (99% water)



about 500-1500 bacteria per sampling location

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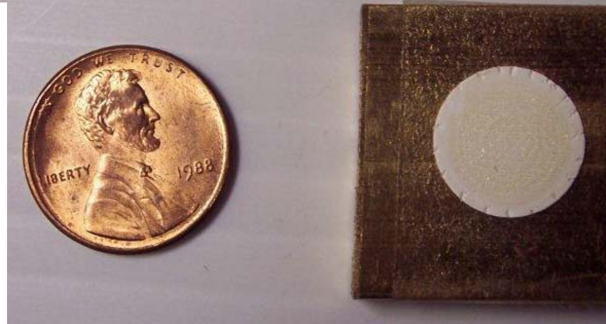
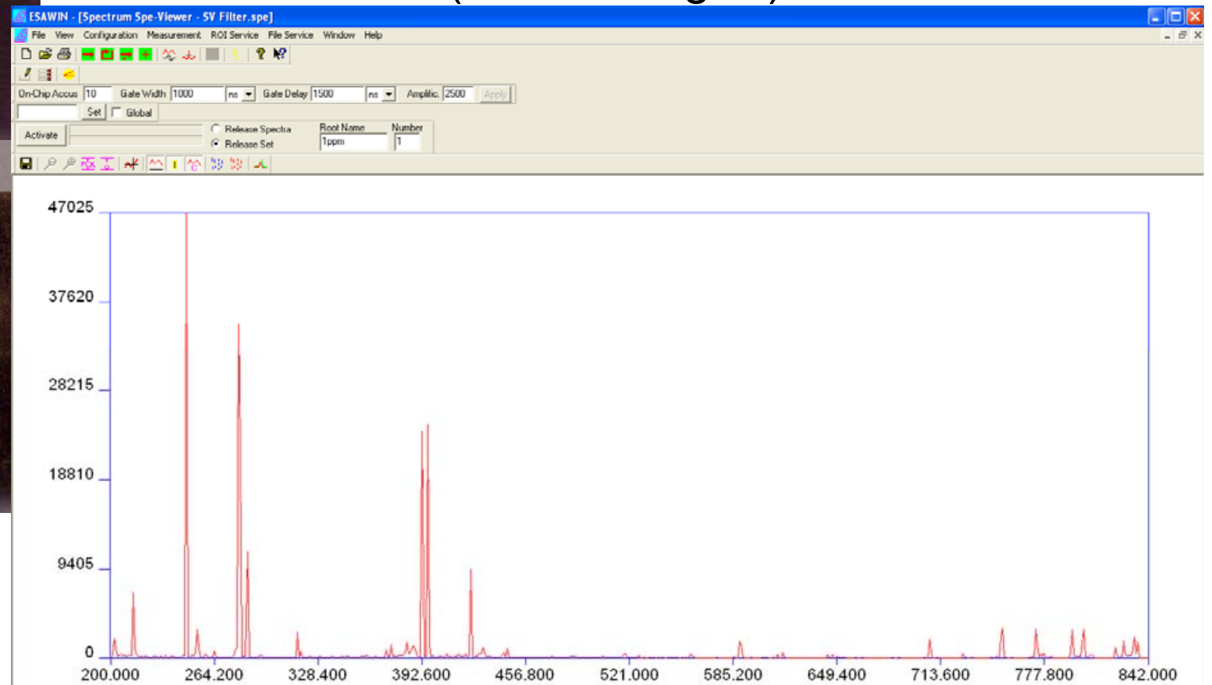
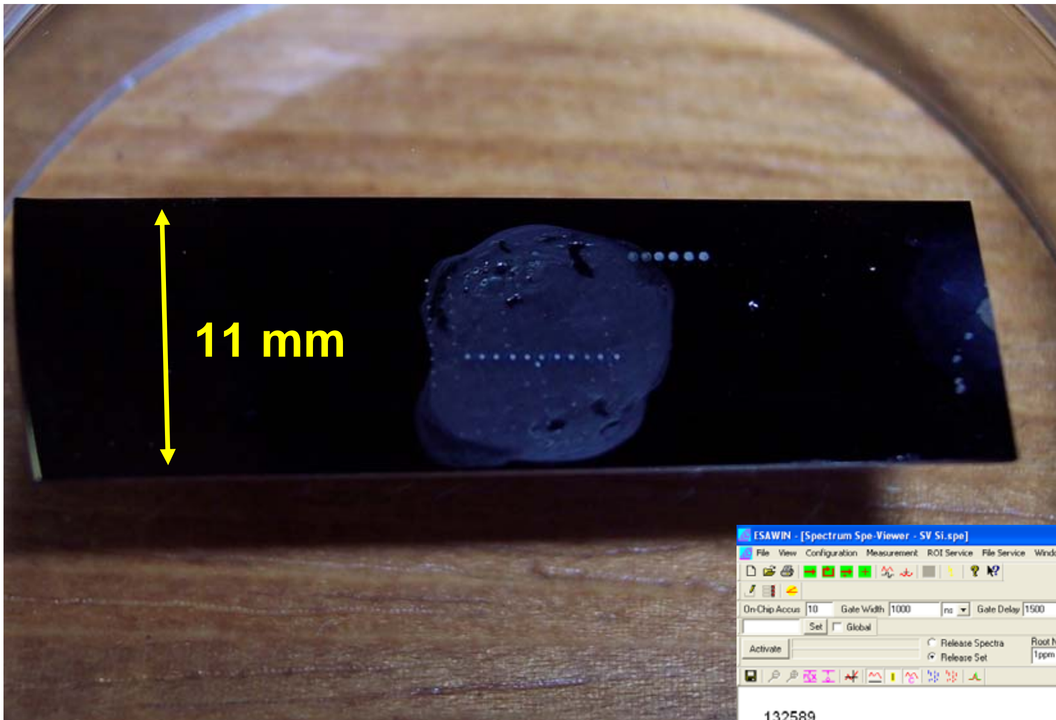
Novel substrates 1



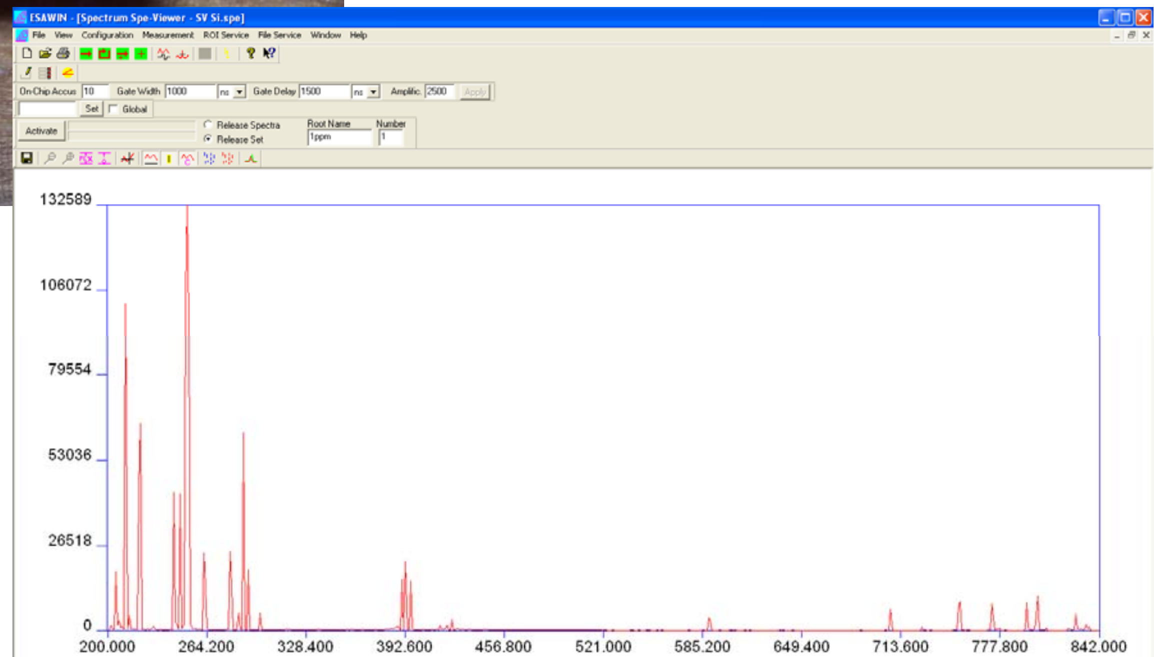
- 10 mL of a suspended bacterial culture pushed through a 0.22 or 0.44 µm cellulose (carbon) Millipore filter
- alternately, bacteria just deposited on filter (wicking)
- C line does “contaminate” spectrum, but only at 7% level (same as agar!)



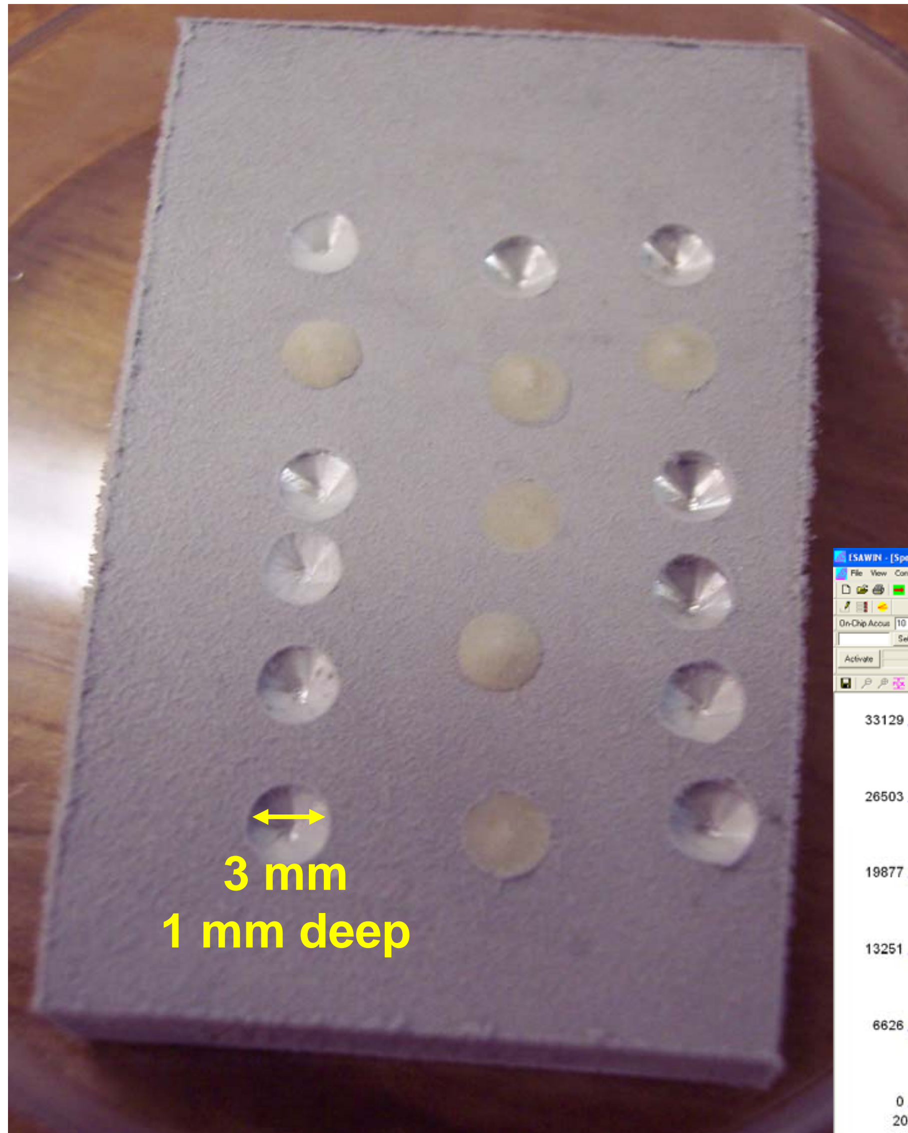
Novel substrates 2



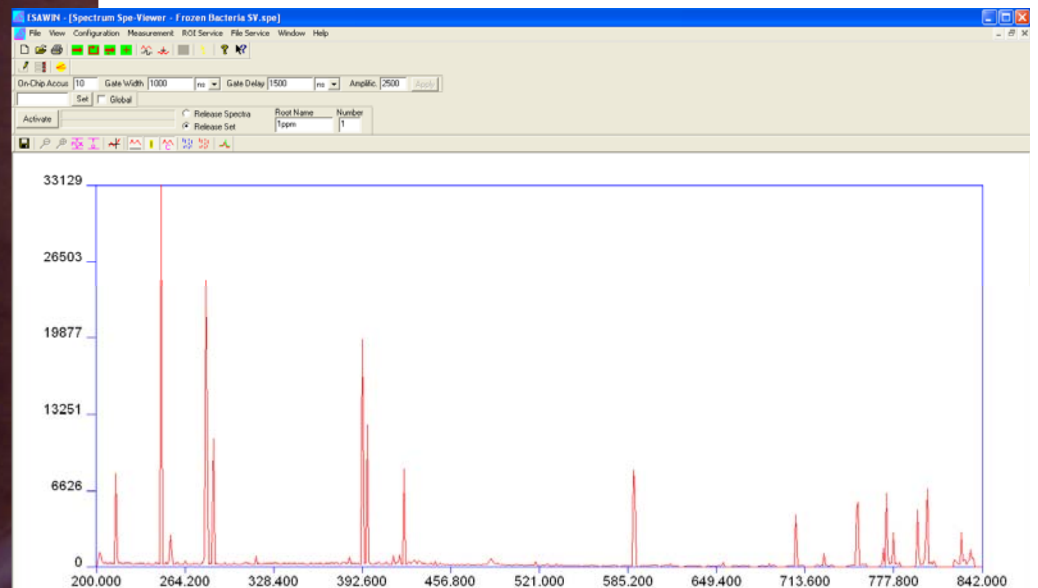
- Acid etched “porous” silicon
- Bacteria fixed with polyacrimide
- High SNR LIBS spectrum
- Si lies do not contaminate spectrum



Novel substrates 3

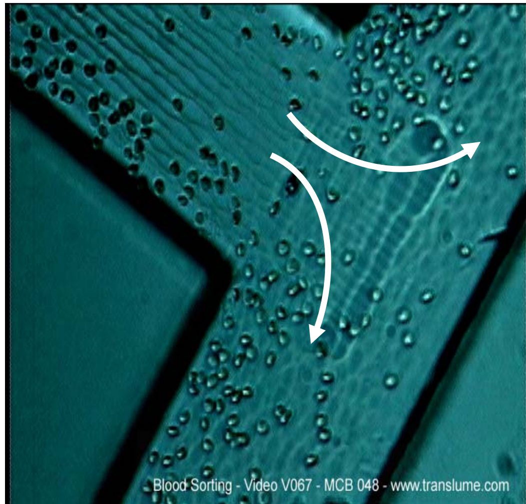


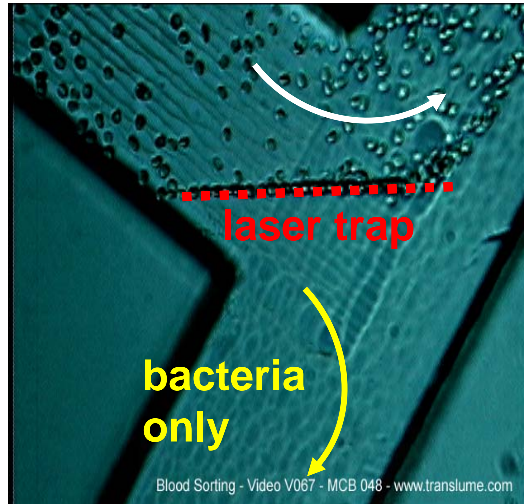
- Bacterial pellets deposited in Al wells
- Frozen with LN2
- High SNR LIBS spectrum
- No contaminating lines



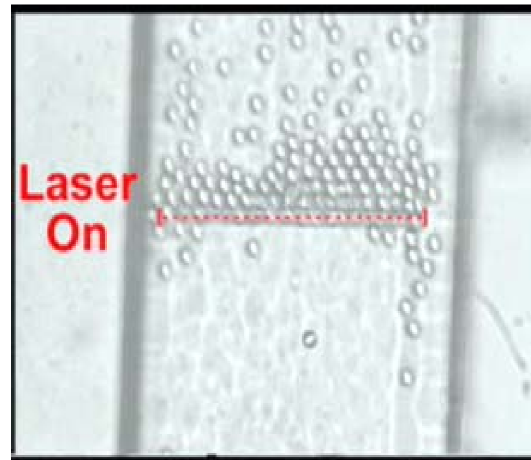
Microfluidic separation/concentration

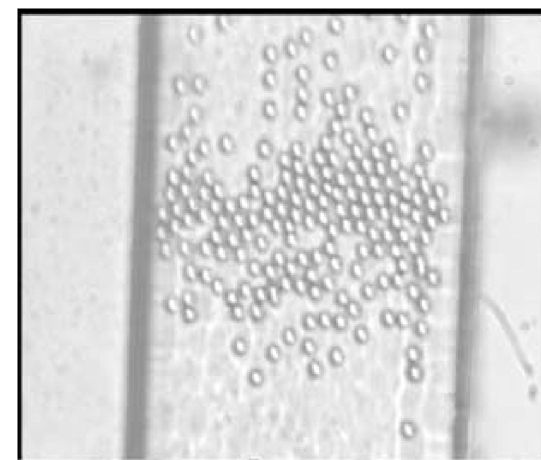
(Translume, Inc. Ann Arbor, MI)





optical trap-based separation of heavier cells from lighter cells





Microfluidic separation/concentration

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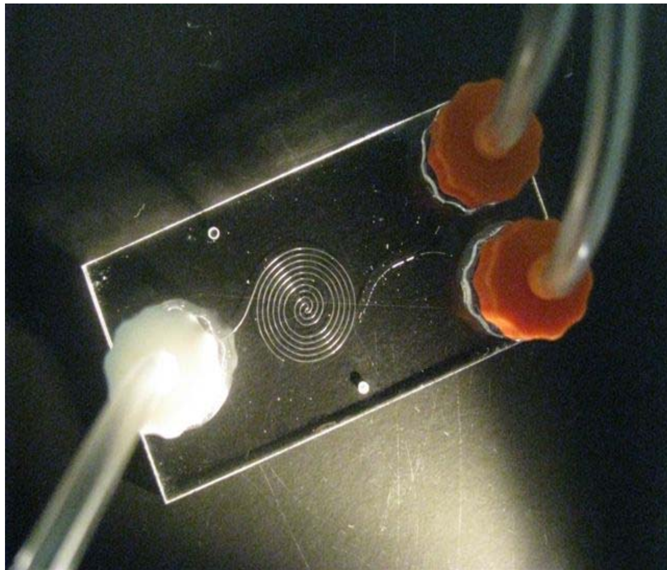
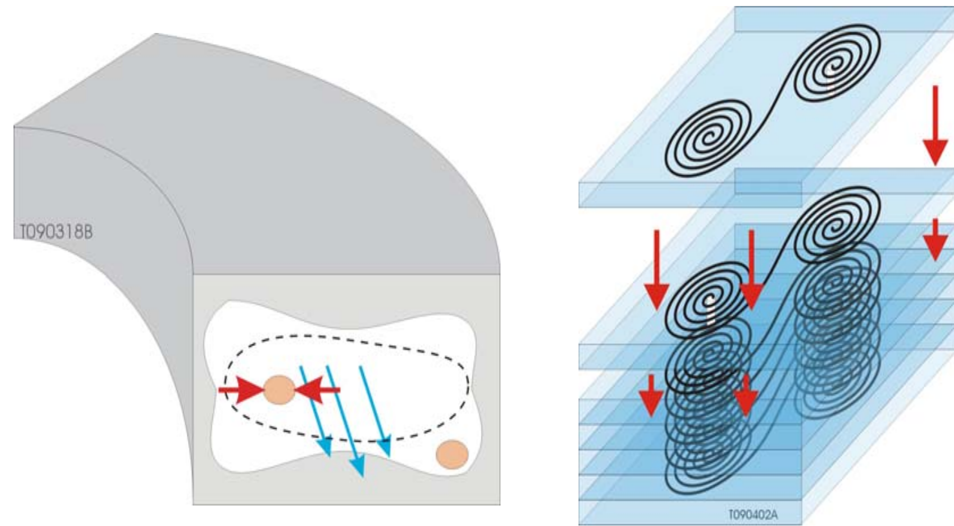


monolithically fabricated devices in glass



hydrodynamic (microfluidic) separation of heavier cells from lighter cells

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Conclusions

- All experiments to date have successfully shown the utility of LIBS to identify bacterial samples in a variety of growth conditions, in mixed samples, in dirty samples, etc.
- We are ready to move to testing real “clinical” type samples through our in-place organizational structure, which combines expertise in hardware development, software development, microbiological handling, and LIBS development.
- The future is approaching quickly. We have got to move forward to stake a claim in 21st Century microbiology.

My group

Thank you…

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