Elemental Multi-variate Microbiological Analysis (EMMA): a Stand-alone Technology and a Sensor-fusion Component for the Rapid Field Identification and Classification of Biomaterials and Biological Agents

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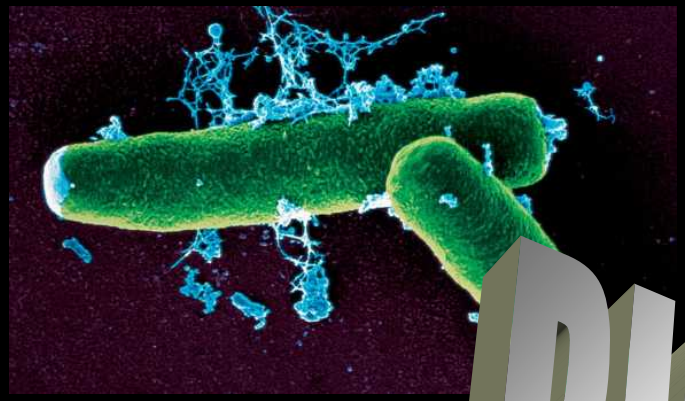
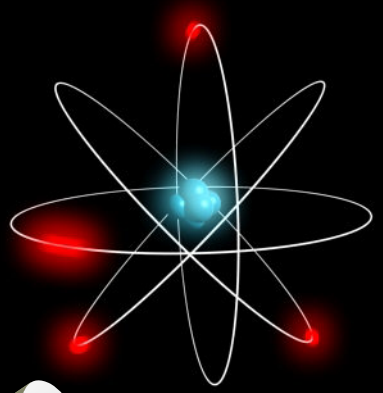
CBD S&T Conference, November 2009, Dallas, TX

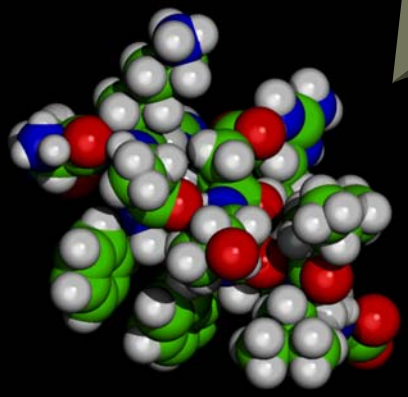
The BIOMAS Project:

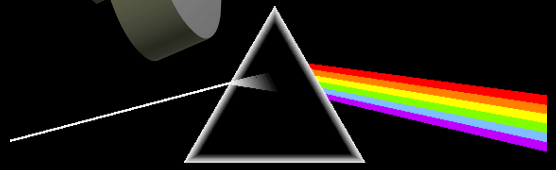
Bacteria Identification by Optical, Molecular, and Atomic Spectroscopy

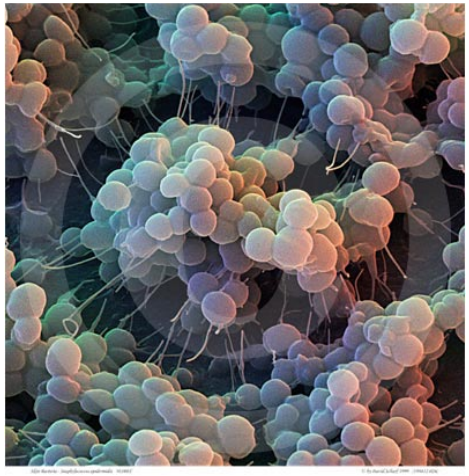




BIOMAS

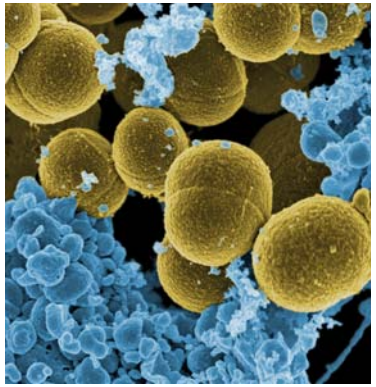






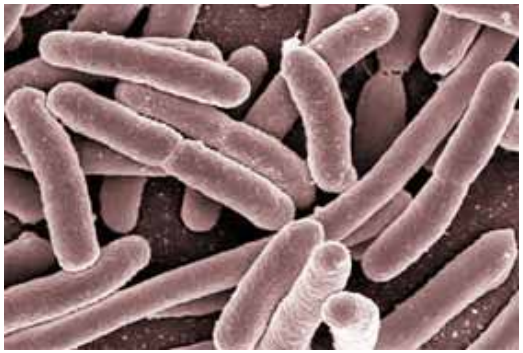
Staph. epidermidis

Staph. aureus

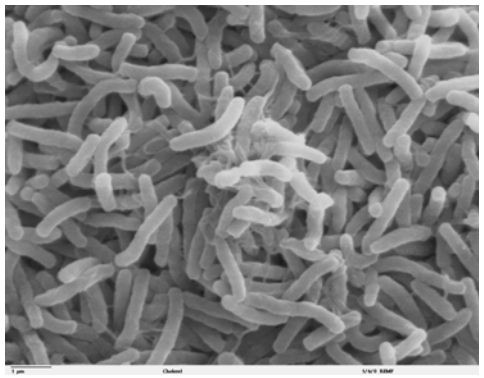


there is an urgent need right now in the military, civilian (hospital, food processing, environmental), and first responder communities for a "…rapid point-of-care (multiplex?) diagnostic for disease-causing pathogens."

E. coli

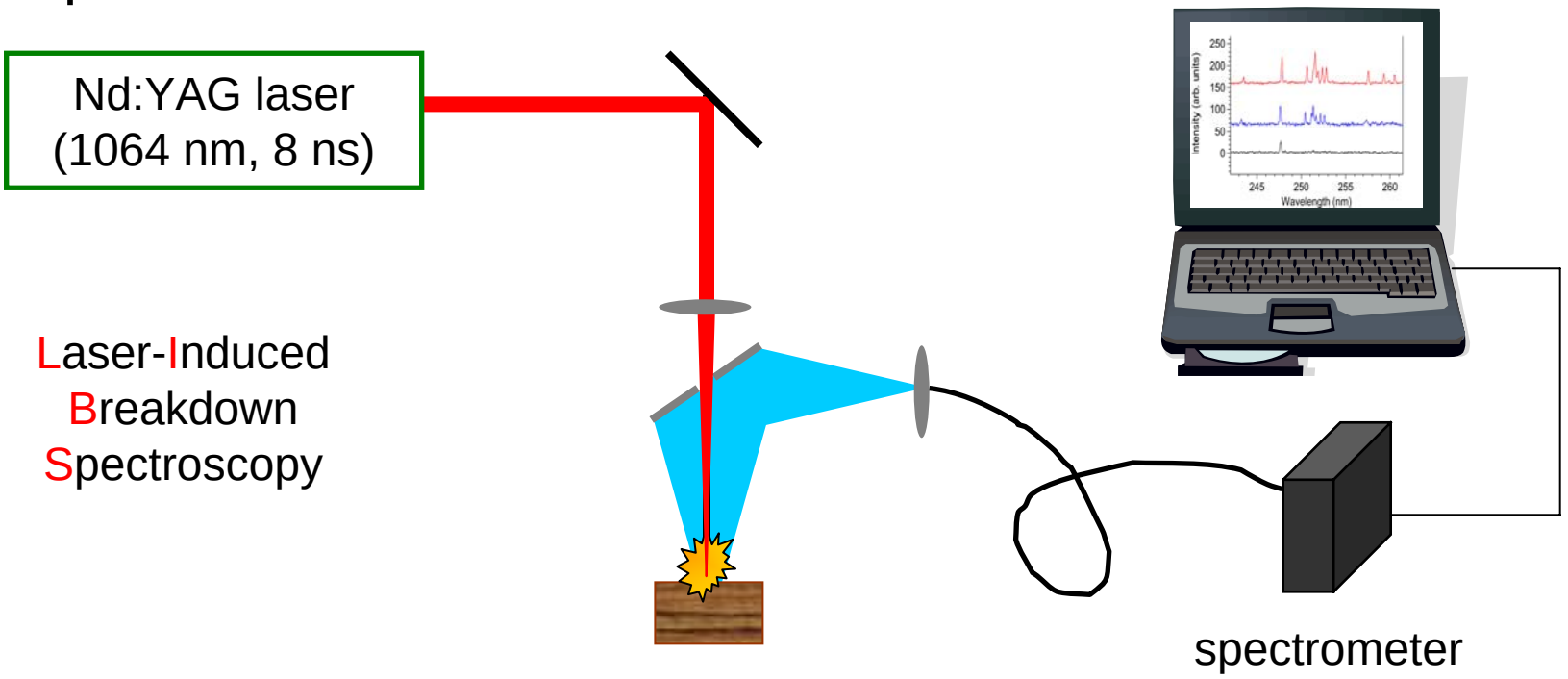


V. cholerae



EMMA: Elemental Multivariate Microbiological Analysis

- utilizes laser-induced breakdown spectroscopy (LIBS) to measure the unique atomic or elemental composition of bacteria



LIBS Spectrum is like a Bar Code- Unique for Each Sample

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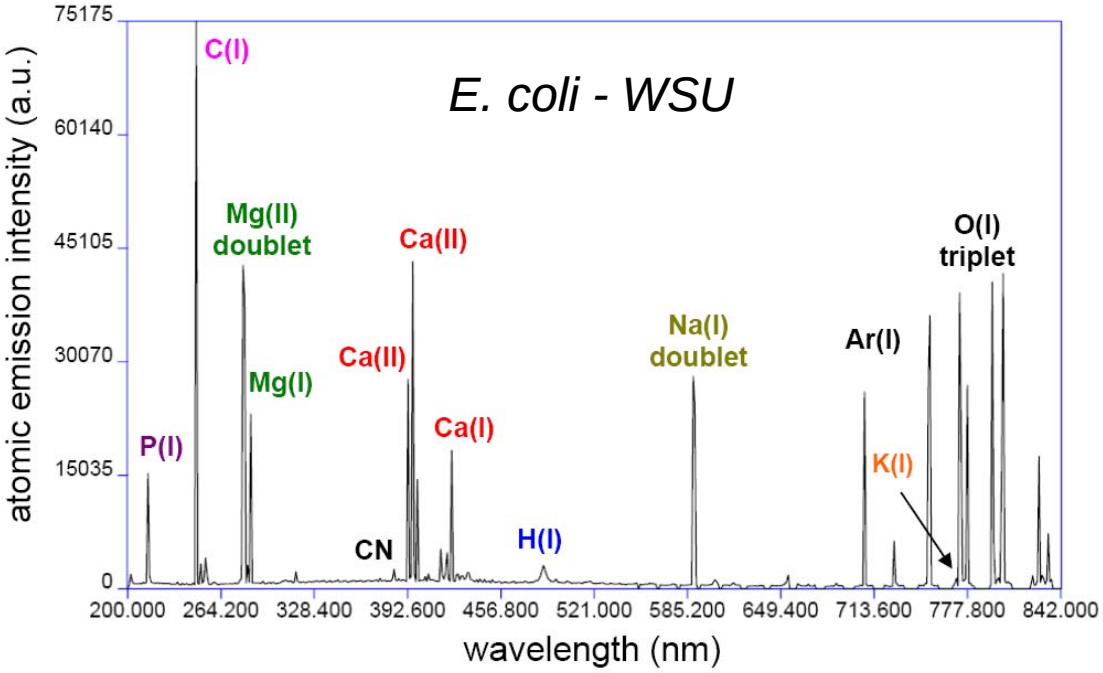

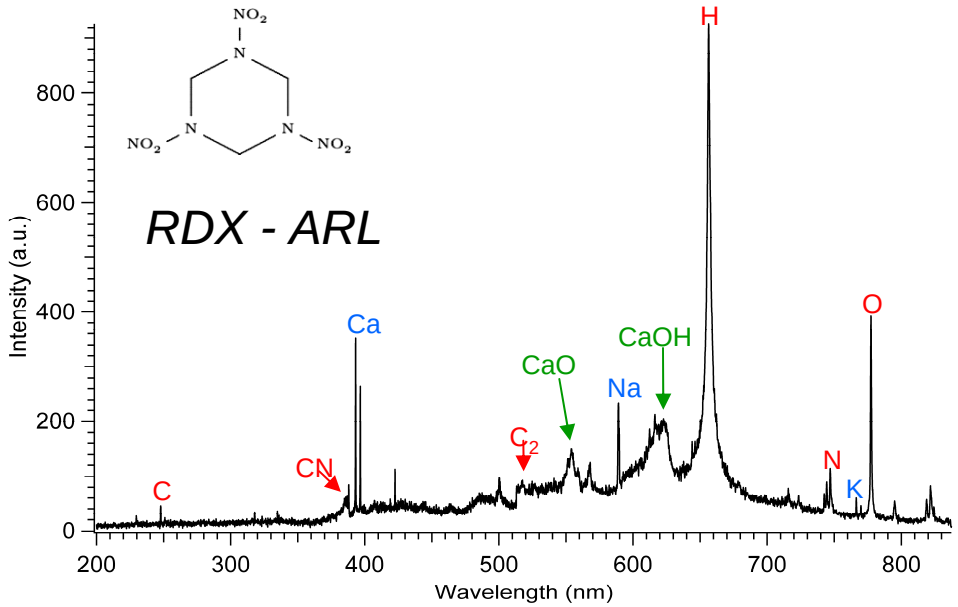

- advanced signal-processing statistical techniques (“chemometrics”) classify/identify the unknown target on the basis of its unique atomic signature
- concentrations of elements (or ratios of concentrations) become independent variables in a chemometric multivariate analysis

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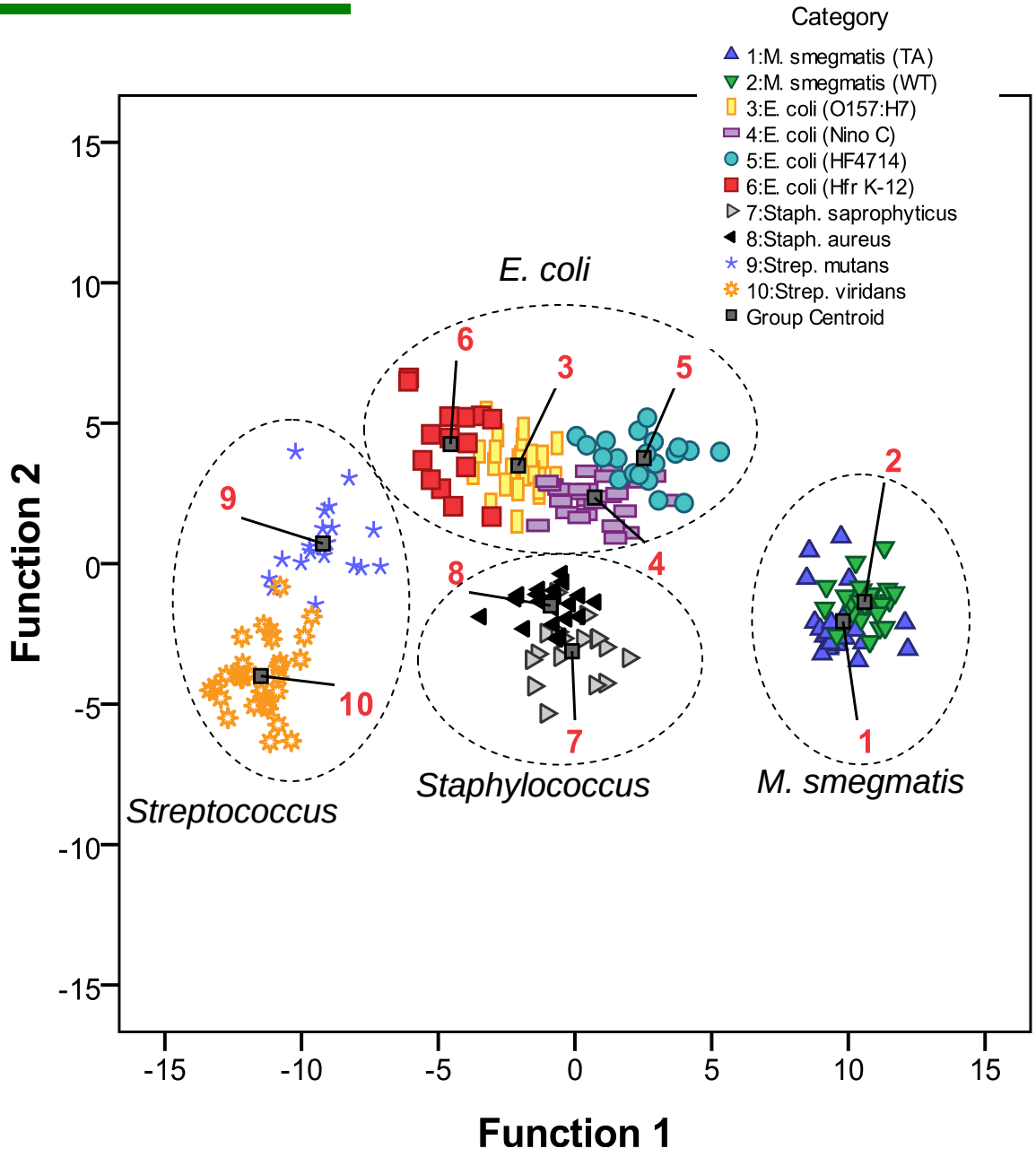
things that make EMMA technology unique

- speed / portability / durability (ruggedness)
  - "rapid point-of-care diagnostic…"
- lack of complicated sample preparation
- no expertise required
- no genetic or antigenic precursors (consumables) necessary
- 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)



The Wayne State Team has already proven…

EMMA spectral fingerprint is:

- growth-medium independent
- independent of state of growth (how “old” the bacteria are)
- independent of whether the bacteria are live or dead
- obtainable even when other types of bacteria or contaminants are present (mixed samples)
- capable of strain discrimination
- obtainable from about 500 bacteria

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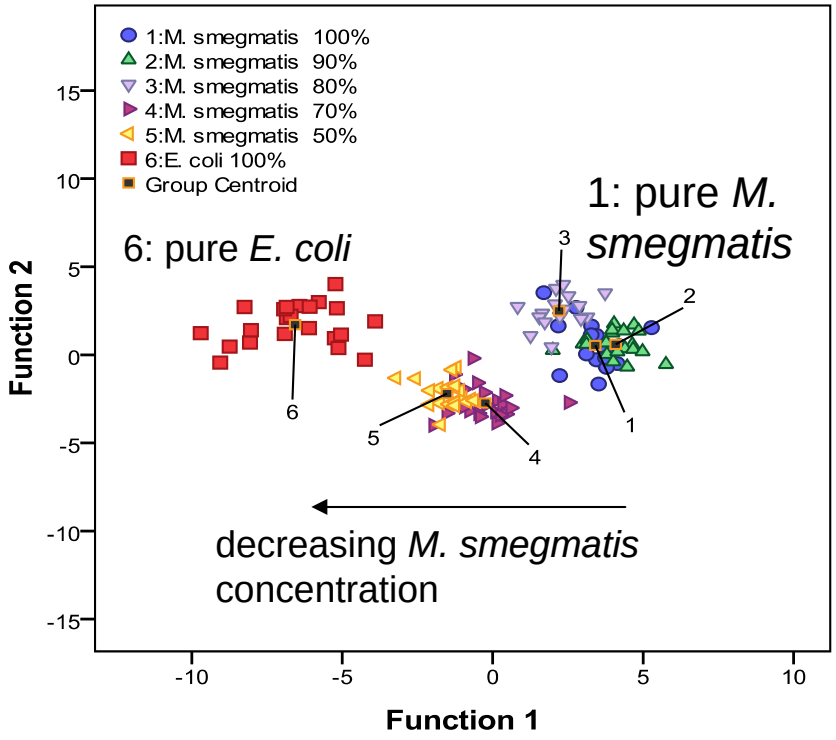
"Mixed" Samples

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%	34%	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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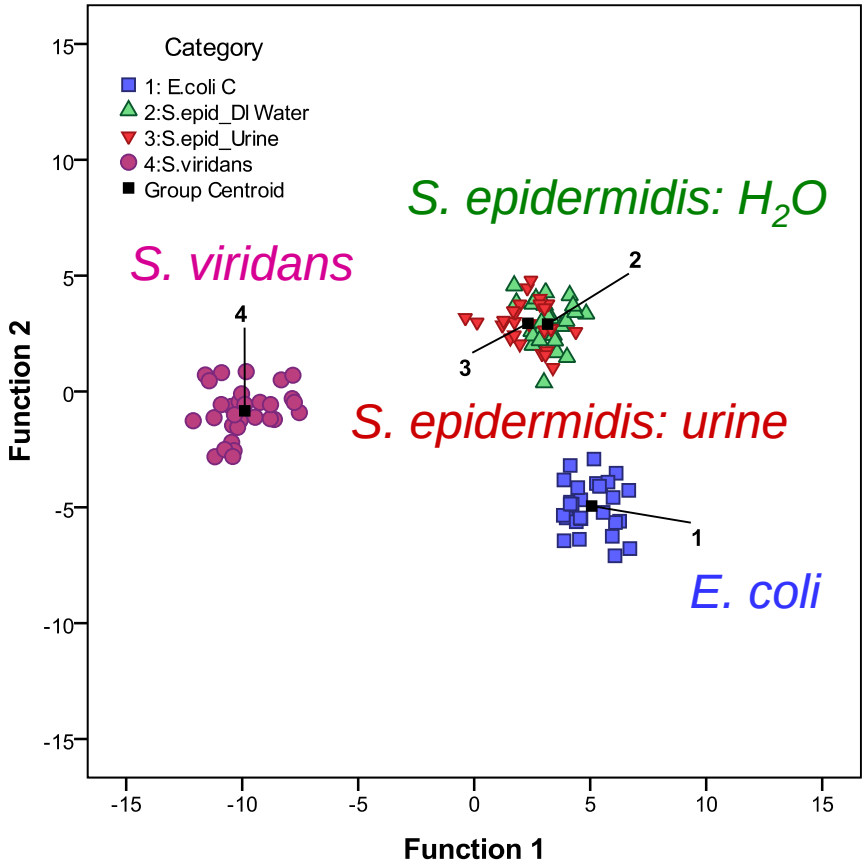
"Dirty" samples



- 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*

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Team

Wayne State University / Detroit Medical Center (micro samples, spectra)

Rehse, Palchaudhuri, Salimnia

Duke University (pattern recognition)

Collins, Torrione

ARL (enhancement, integration)

LIBS Group

Translume, Inc. (sample prep. front end)

Haddock

Firm (device design and construction)

to be determined

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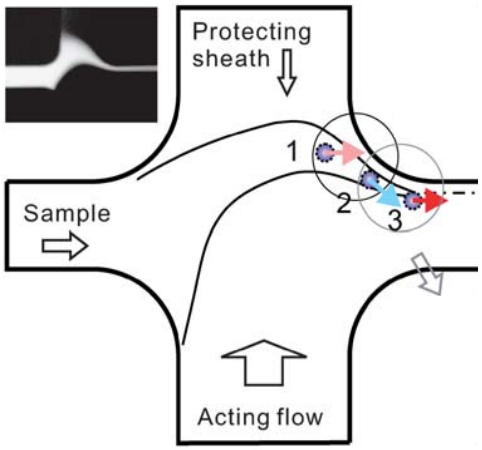
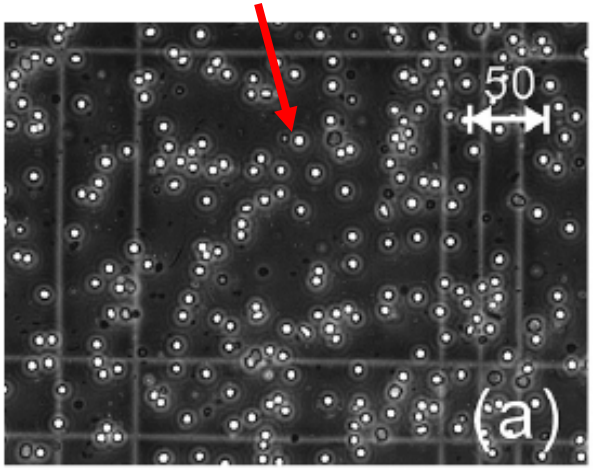
Microfluidic separation/concentration



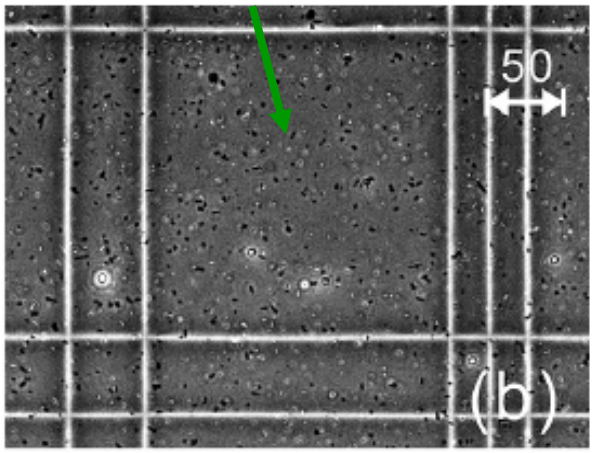
A "lab on a chip" microfluidic design by Wu et al. that has been shown to separate *E. coli* from red blood cells in human blood flow resulting in bacterial concentration

red blood cells



sample pre-separation

E. coli



sample after separation / concentration

Soft inertial microfluidics for high throughput separation of bacteria from human blood cells

Zhigang Wu,*[a] Ben Willing,[b] Joakim Bjerketorp,[b] Janet K. Jansson[bc] and Klas Hjort[a]

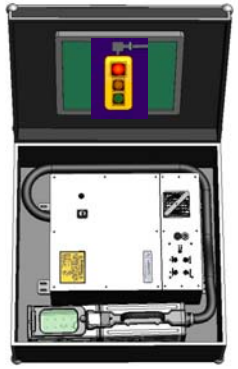
Lab Chip, 2009, **9**, 1193–1199

Field-Portable Hardware

An example of current commercial LIBS hardware that operates on wallplug power

Applied Photonics Limited

Under development: battery operated for rapid field analyses



- eye-safe

- choice of sample chambers (smaller/larger)

- real-time chemometrics

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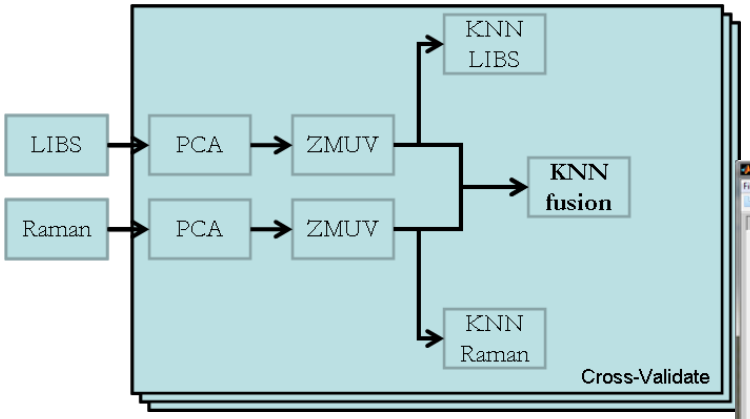
Sensor Fusion (with Raman)

- Team at Duke are experts in real-time chemometric analysis
  - under US Army contract, delivered the very first truly “real-time” software for analysis of LIBS spectra (identified biological warfare simulant in one second!)

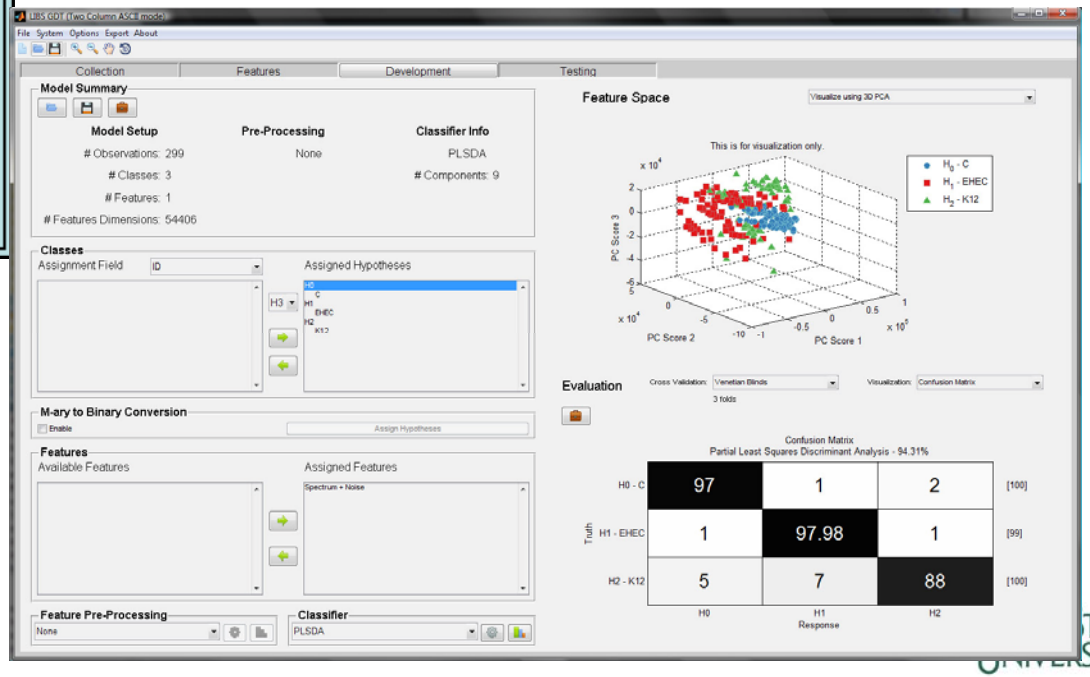
- Also working with us on LIBS/Raman data fusion

“Data Level” Sensor Fusion

- Flow chart below shows example of Data-Level-Fusion, explored for PCA, LDA, PLS on data from three *E. coli* strains.

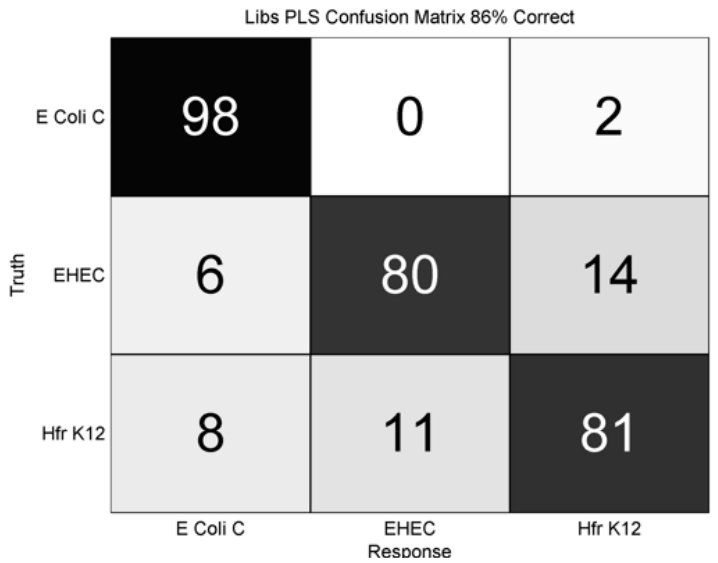


New Folder Consulting

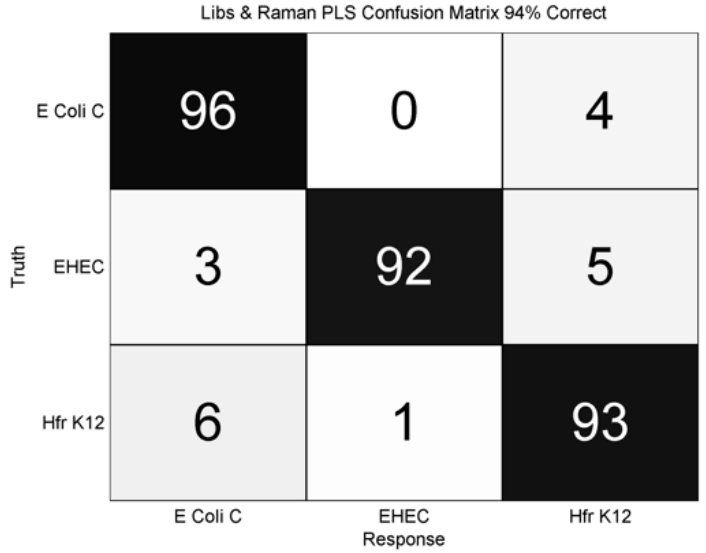


LIBS/Raman Data fusion with a PLS

- LIBS: 86%



- **Fusion: 94% correct *E. Coli* identification**



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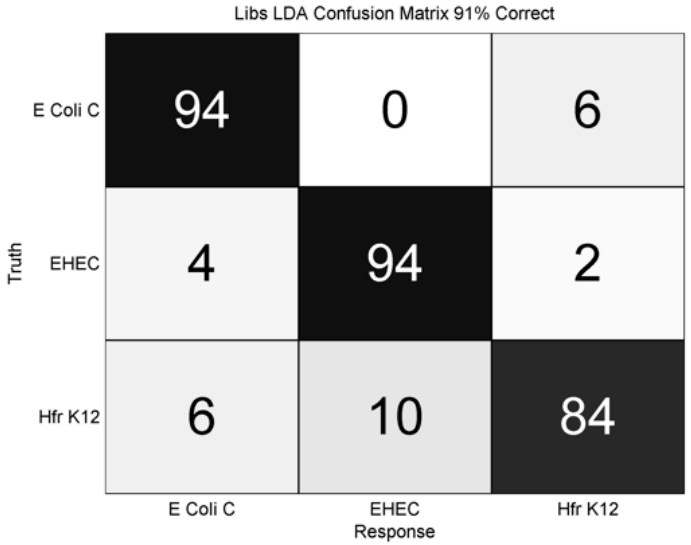
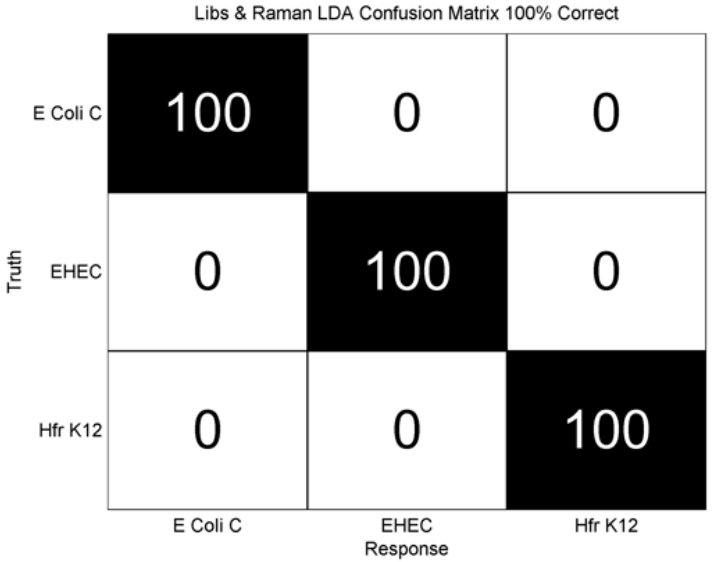
LIBS/Raman Data fusion with a LDA

- LIBS: 91%



- **Fusion: 100% correct *E. Coli* identification**



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Conclusions

- All EMMA 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

- Early result show LIBS can be combined with Raman for improved accuracy of identification: “sensor fusion.”