The Future of LIBS-Based Pathogen Identification

Steven J. Rehse[1], Qassem Mohaidat[2], Sunil Palchaudhuri[3], Hossein Salimnia[4]

[1]The University of Windsor, Department of Physics

[2]Wayne State University, Department of Physics & Astronomy
[3]Wayne State University, Department of Immunology & Microbiology
[4]Detroit Medical Center University Laboratories

There are plenty of advanced ideas (especially at FACSS/SCIX) to rapidly identify pathogenic bacteria optically.

And yet…

Cantaloupe deaths and illnesses expected to rise

Federal health officials said Wednesday more illnesses and possibly more deaths may be linked to an outbreak of *listeria* in cantaloupe in coming weeks.

So far, the outbreak has caused at least 100 illnesses — including up to 18 deaths* — in 18 states, making it the deadliest food outbreak in the United States in more than a decade.

*CDC, Wednesday

University of Windsor
thinking forward

What's Lurking in Your Meat and Poultry? Probably Staph

Researchers were surprised to find that nearly half of samples of beef, pork and poultry tested from popular grocery stores were contaminated with *Staphylococcus aureus*, a bacteria that the USDA doesn't even monitor in the food source, because it's not known as a common food-borne pathogen. And of the bacteria found, nearly all were strains that were resistant to more than one antibiotic

The History of LIBS-Based Pathogen Identification

University of Windsor
thinking forward

2003-2004 **early days** **feasibility; proof of concept**

Samuels, DeLucia, Jr., Morel, Leone, Amoroux, Miziolek, Harmon, Hybl, Buckley

2005-2008 **advanced days**

Baudelet, Wolf, Laloi, Gottfried, Dixon, Hahn

advanced chemometrics; single particle/bioaerosals; double pulse; femtosecond; use of molecules; stand-off; man-portable

2008-2011 **current days**

Multari & Cremers, Caceres & Marcos-Martinez, Baudelet, Rehse & Mohaidat

discrimination of strains; microbiological diversity to simulate clinical specimens; realistic tests; chemometrics.

Future Days…

2011-? **future days**

testing of ever greater numbers of bacterial species;

testing of clinical specimens;

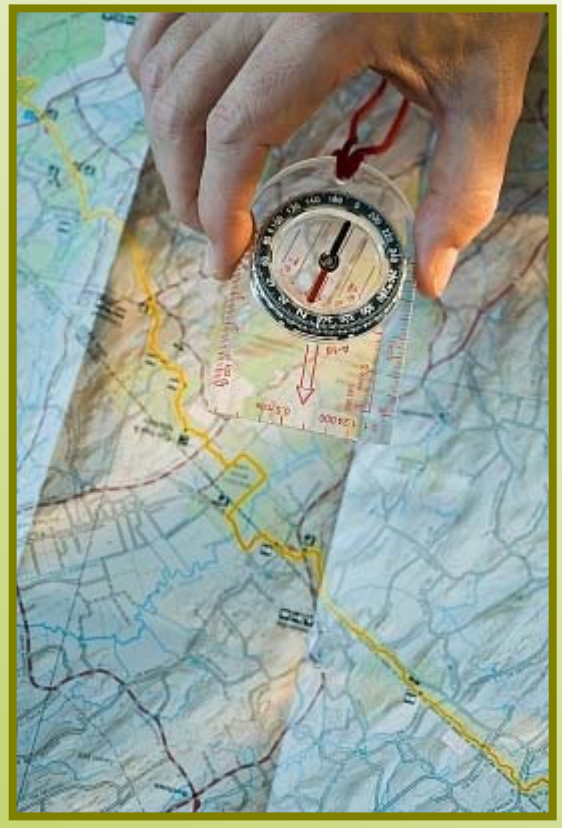
translation of technology to clinical medicine;

commercial benchtop instruments;

***in vivo* measurements**

Why Do I Think This?

Based on where we are now…

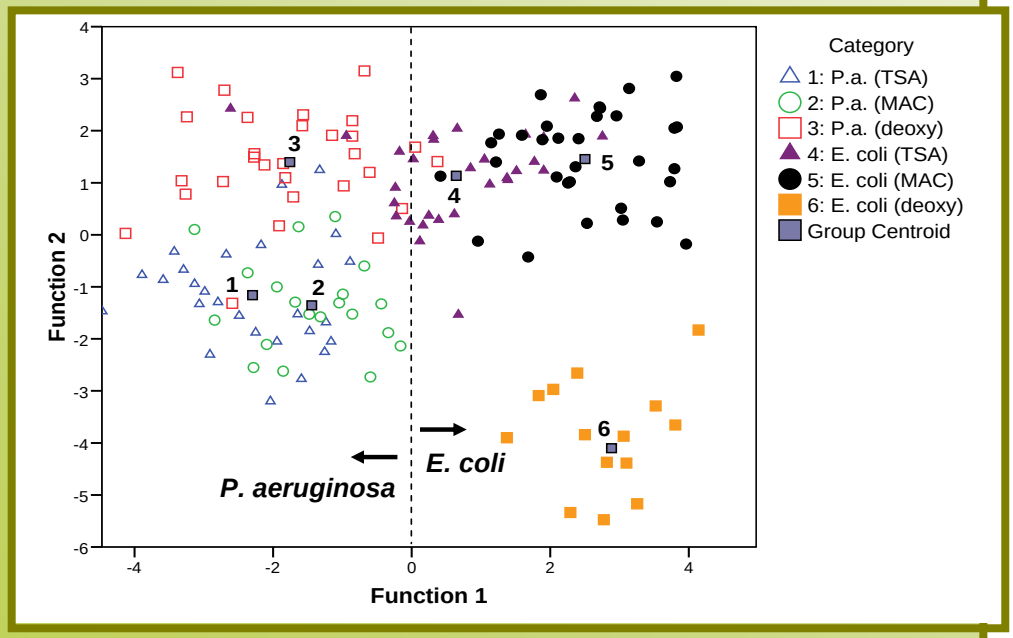


…past trajectory and current position indicate the way forward

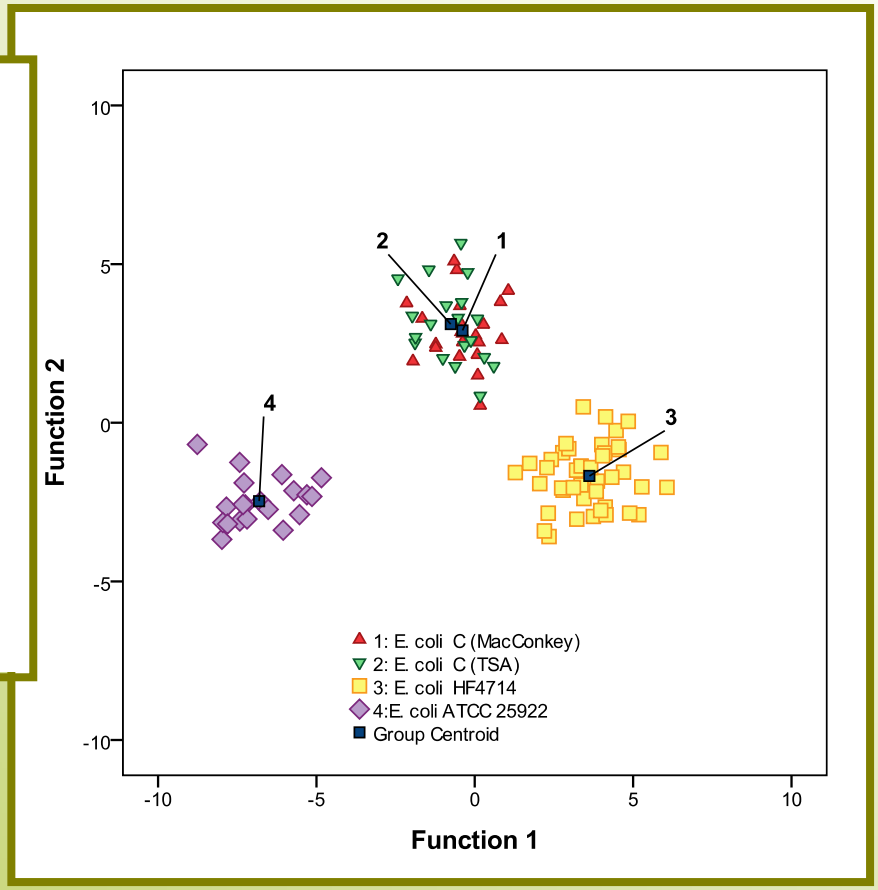
You are not what you eat (if you are a bacterium)

growth medium tests

2009



2011



Confirmation by Caceres Group

Talanta 84 (2011) 730–737

Identification and discrimination of bacterial strains by laser induced breakdown spectroscopy and neural networks

D. Marcos-Martinez [a], J.A. Ayala [b], R.C. Izquierdo-Hornillos [a], F.J. Manuel de Villena [a], J.O. Caceres [a,*]

[a] *Departamento de Química Analítica, Facultad de Ciencias Químicas Universidad Complutense, 28040 Madrid, Spain*
[b] *Centro de Biología Molecular "Severo Ochoa", CSIC, C/Nicolás Cabrera, 1, Cantoblanco, 28049 Madrid, Spain*

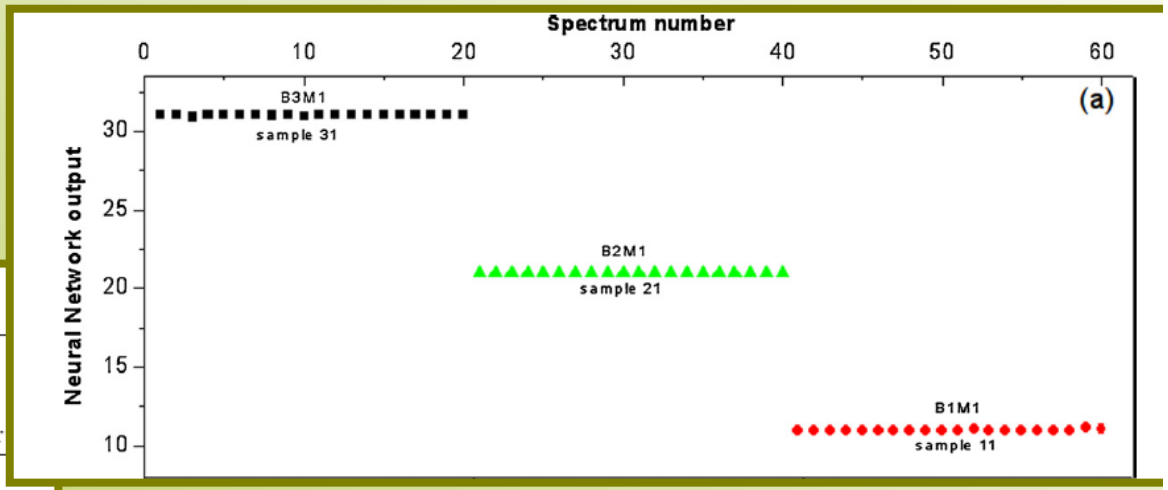
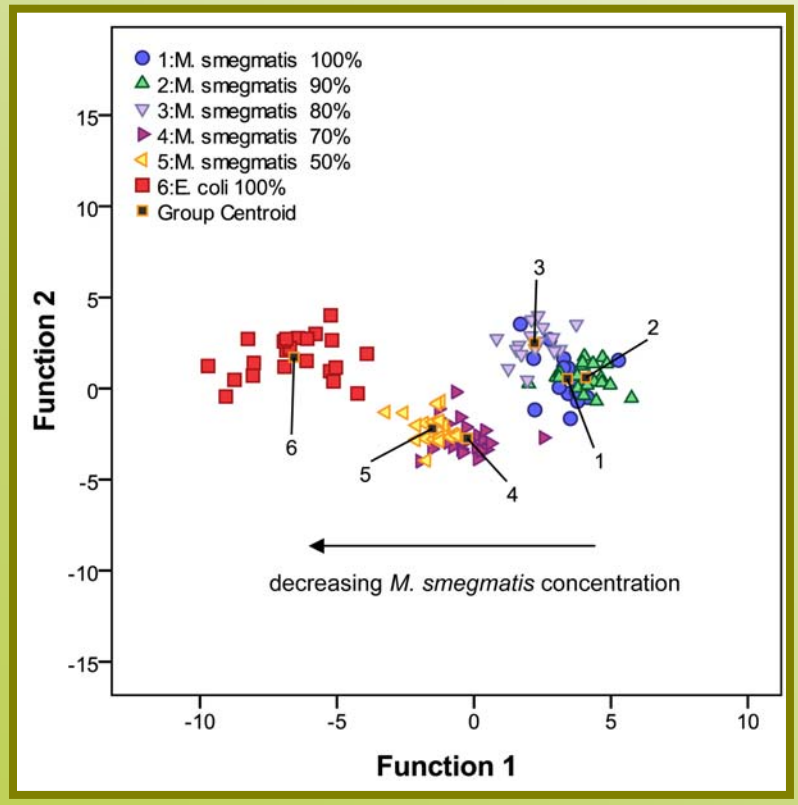


Table 1
Nomenclature used for the samples.

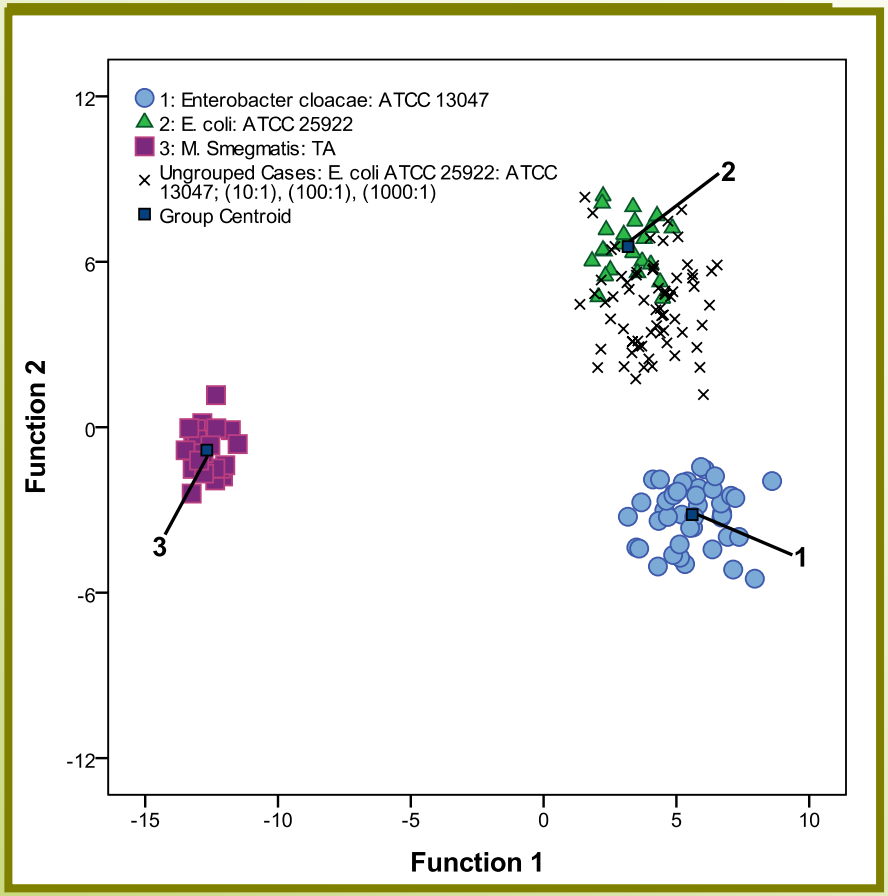
Bacterial strains	Culture media 1 LB agar	Culture media 2 MacConkey agar	Culture media 3 Brucella anaerobic agar
Pseudomonas aeruginosa (B1)	B1M1 (11)	B1M2 (12)	B1M3 (13)
Escherichia coli (B2)	B2M1 (21)	B2M2 (22)	B2M3 (23)
Salmonella typhymurium (B3)	B3M1 (31)	B3M2 (32)	B3M3 (33)

Contamination of samples will not degrade specificity

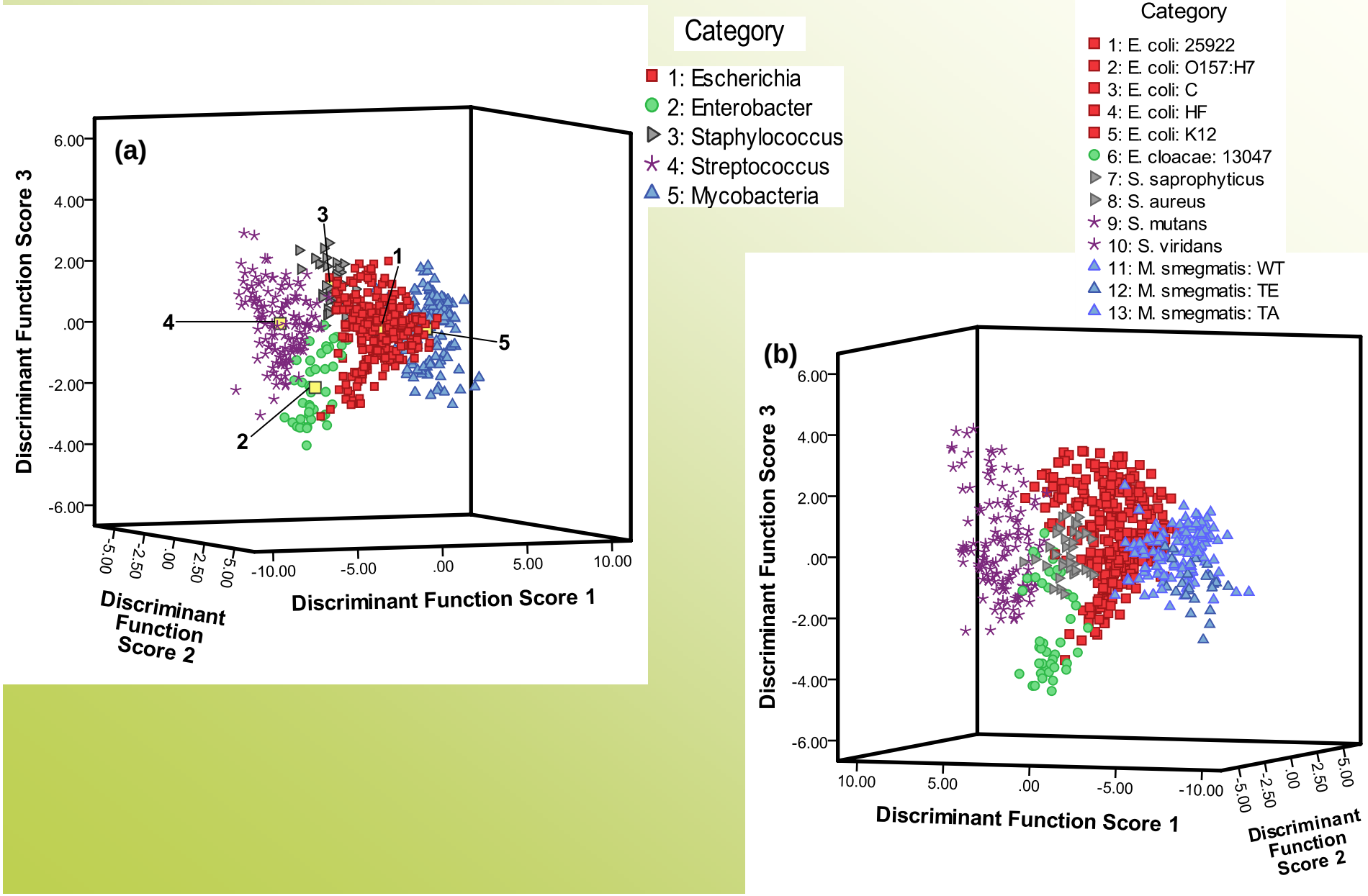
2010



2011



Selectivity: A five genus test



Selectivity: A five genus test

truth tables for external and cross validation

DFA classification of 669 bacterial LIBS spectra (acquired over 3 years) in a five-genus DFA model.

External validation

Escherichia	TRUE	FALSE
Positive	89.97%	4.28%
Negative	95.72%	10.03%

Staphylococcus	TRUE	FALSE
Positive	62.16%	2.55%
Negative	97.45%	37.84%

Streptococcus	TRUE	FALSE
Positive	83.82%	2.04%
Negative	97.96%	16.18%

Mycobacterium	TRUE	FALSE
Positive	89.61%	1.27%
Negative	98.73%	10.39%

Cross validation (LOO)

Escherichia	TRUE	FALSE
Positive	94.31%	0.61%
Negative	99.39%	5.69%

Staphylococcus	TRUE	FALSE
Positive	100.00%	0.51%
Negative	99.49%	0.00%

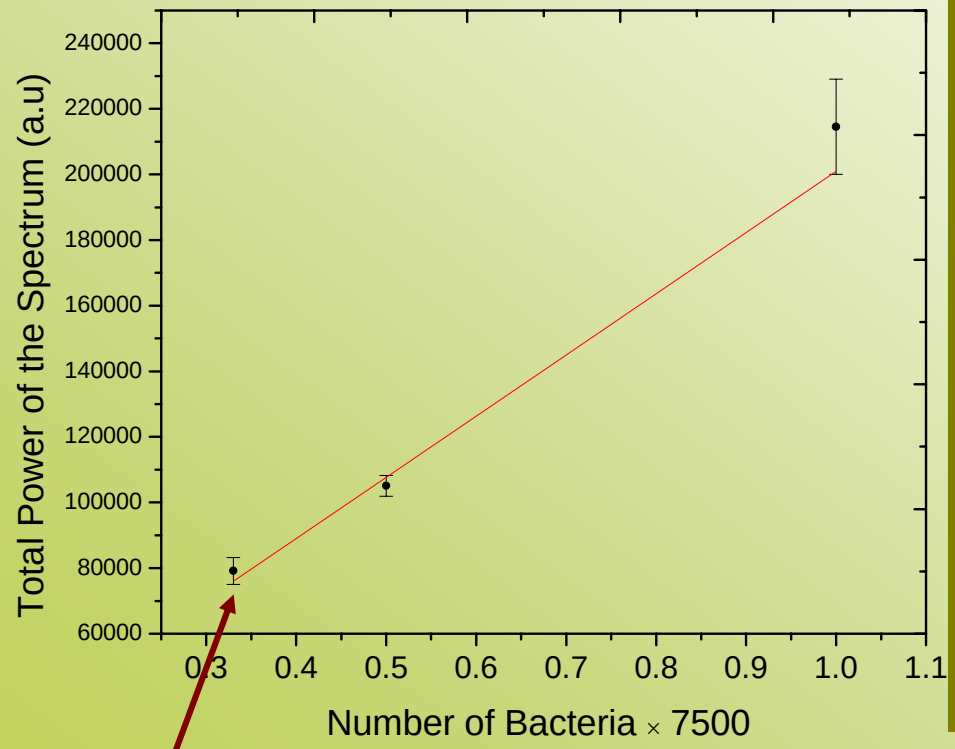
Streptococcus	TRUE	FALSE
Positive	95.59%	0.00%
Negative	100.00%	4.41%

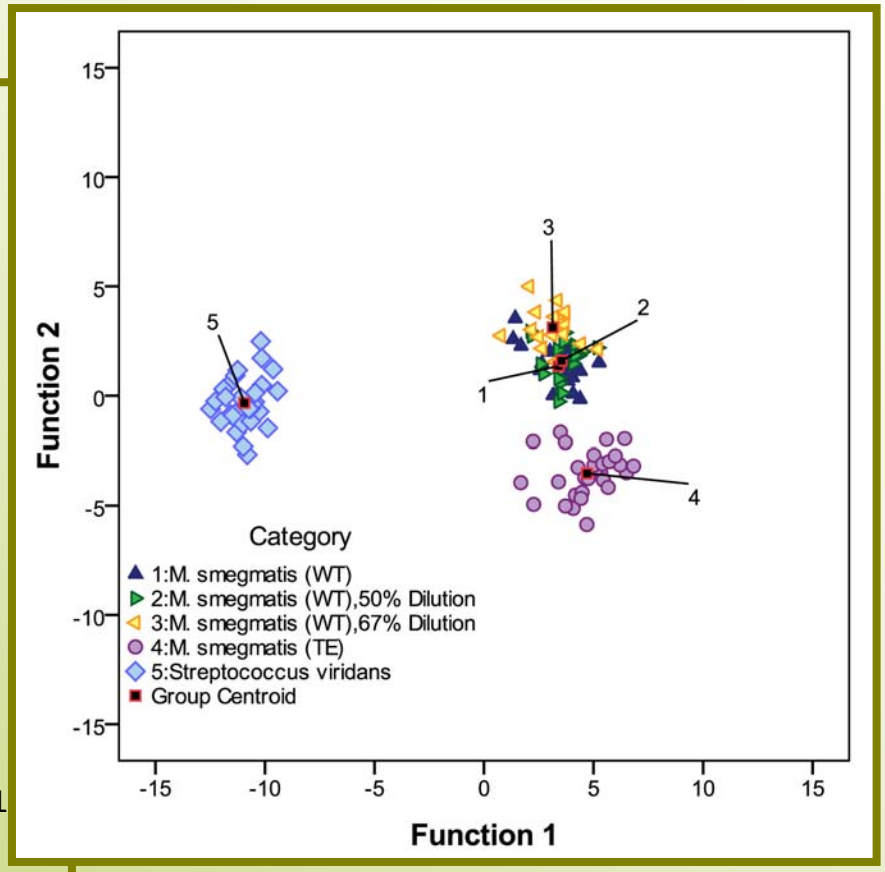
Mycobacterium	TRUE	FALSE
Positive	97.40%	0.00%
Negative	100.00%	2.60%

Dilution

specimens of various titer

2010



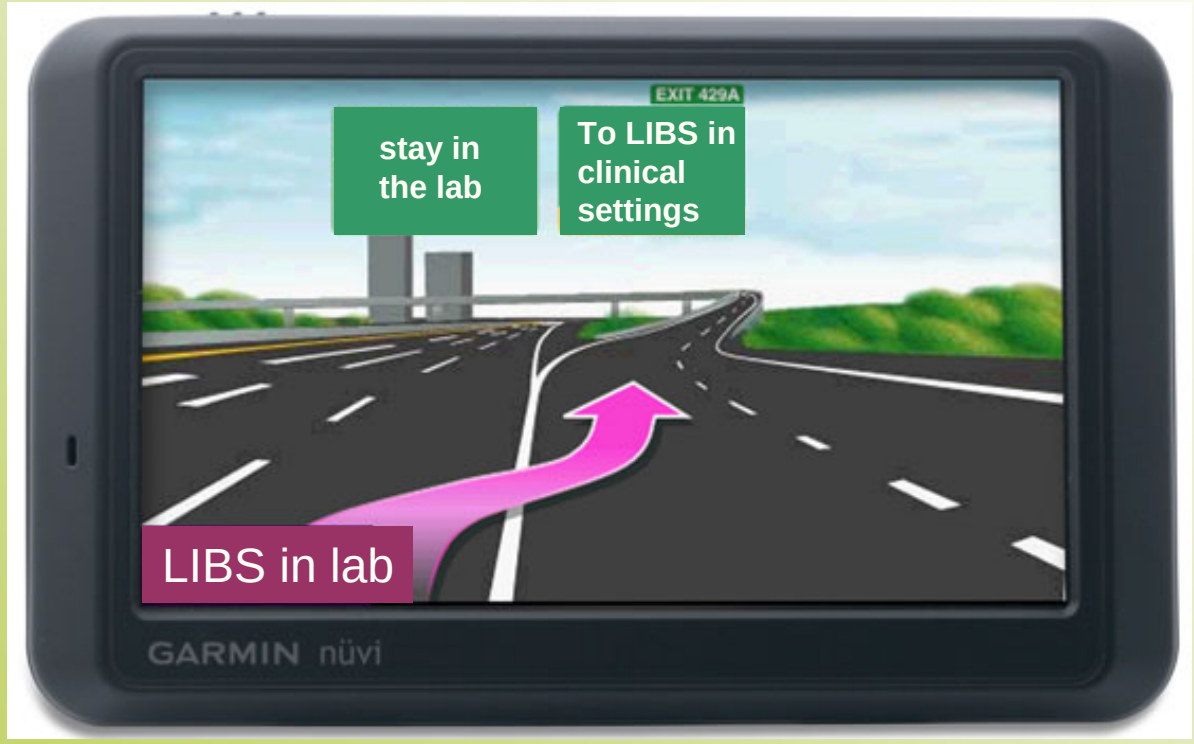


5 laser sampling locations

~500 bacteria per locations

Where Should We Go Now?

"Recalculating…"



Where I Think We Should Go

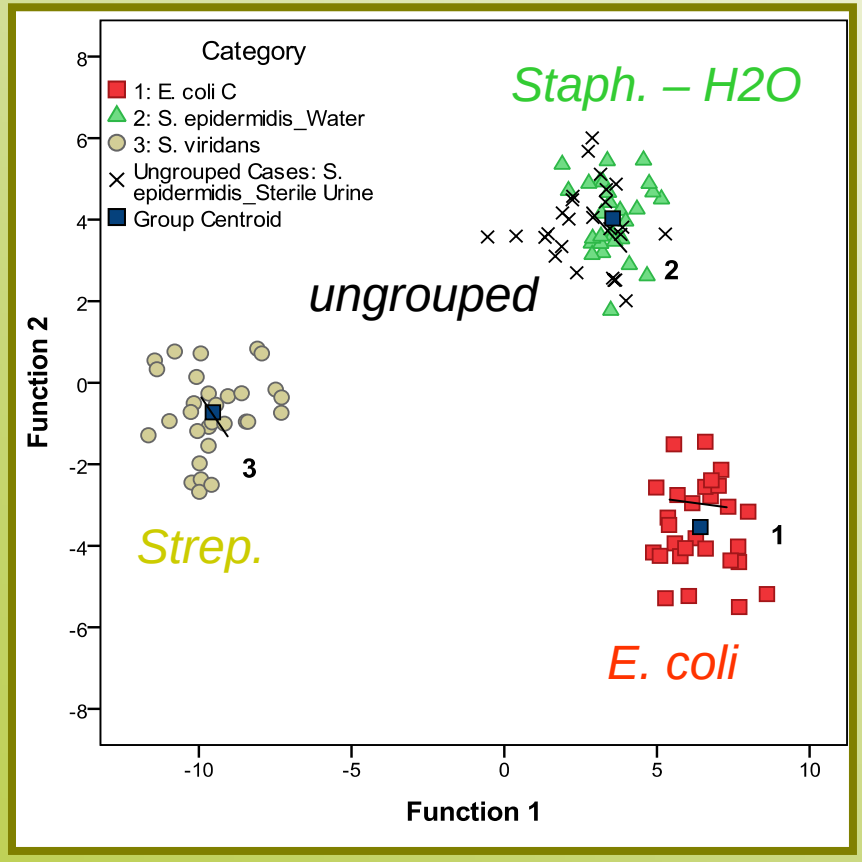
(1) Clinical specimens that should be normally sterile and contain minimal other cellular components (i.e. urine, cerebral spinal fluid)

- detect the presence of bacteria
- make a rapid classification of that bacteria.

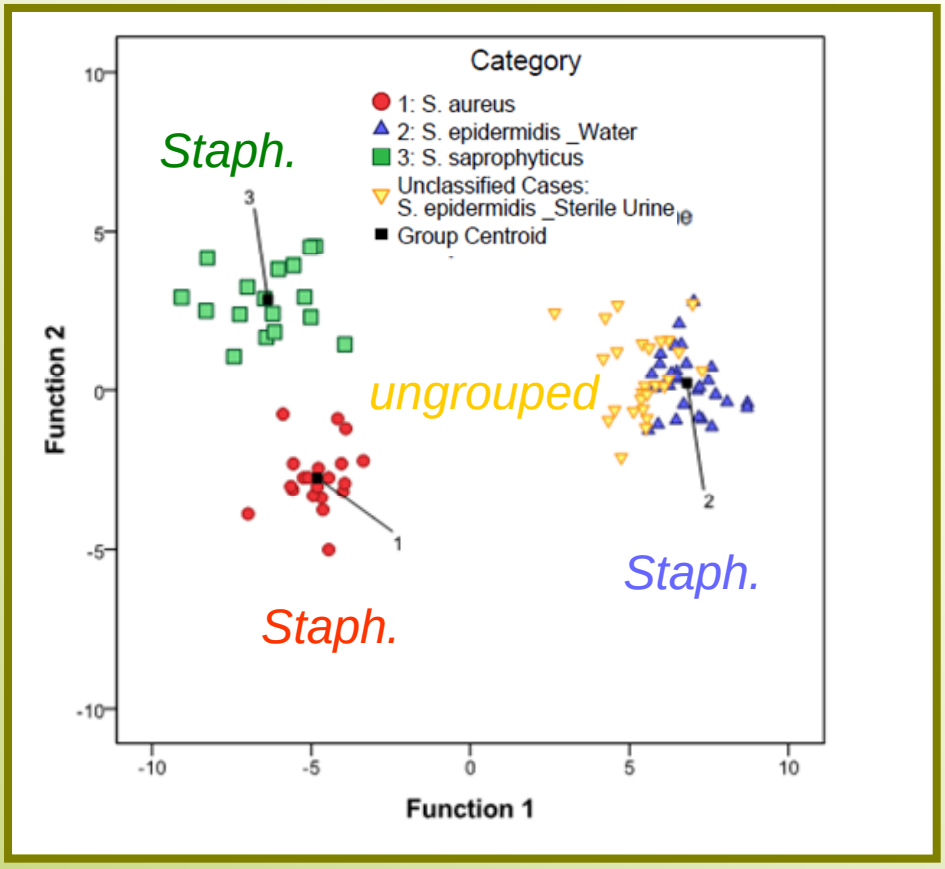
Simulated Clinical Specimens:

S. epidermidis in sterile urine

2011



2011



Where I Think We Should Go

(1) Clinical specimens that should be normally sterile and contain minimal other cellular components (i.e. urine, cerebral spinal fluid)

- detect the presence of bacteria
- make a rapid classification of that bacteria.

(2) Strain classification (particularly antibiotic-resistant pathogen strains such as MRSA).

These two applications alone (MRSA infections and UTI's) are responsible for over $2 billion of medical costs worldwide every year.

Most deaths from meningitis occur in less than a day from onset of the fever. It is most commonly caused by one of three types of bacteria: *Haemophilus influenzae*, *Neisseria meningitidis*, and *Streptococcus pneumoniae*.

Long-Term Objectives

(1) LIBS-based pathogen identification must be applicable to blood samples.

- The cellular components of blood?
- More complex sample-preparation steps for bacterial separation and identification needed.
- New sample-handling techniques needed.
- Advances made in the application of LIBS to liquid samples should be integrated to allow the rapid testing of the bacteria in fluid media.

(2) In all cases, efforts should now be made to include clinical collaborators.

- Allows the testing of clinical specimens in blind tests.
- All results initially confirmed by more traditional but rigorous microbiological (genetic and molecular microbiology) methods.

(3) Results published in medical journals.

Motivation of Long-Term Objectives

- Only in this way will the technique gain acceptance and the required traction in the medical community.
- We've got an important story to tell; let's tell it!

University of Windsor
thinking forward

Thank you.