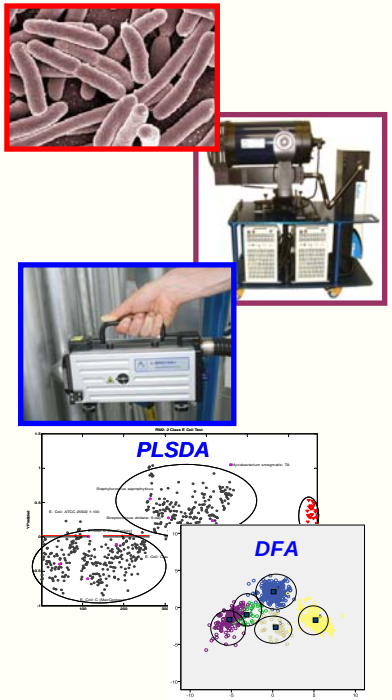
Recent advances in the use of laser-induced breakdown spectroscopy (LIBS) as a rapid point-of-care pathogen diagnostic

presented at the 2013 CAP Congress
Montreal, QC May 2013



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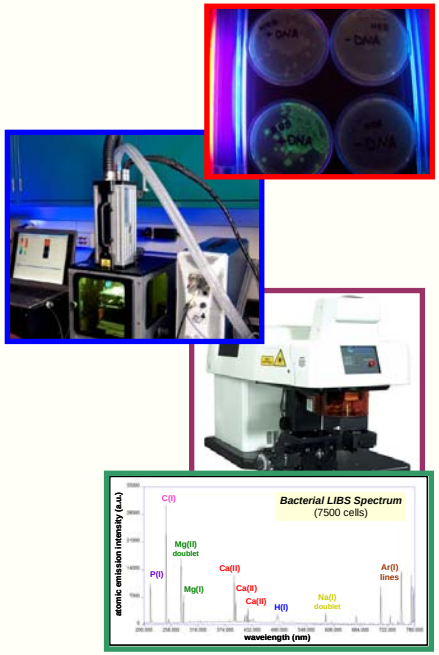

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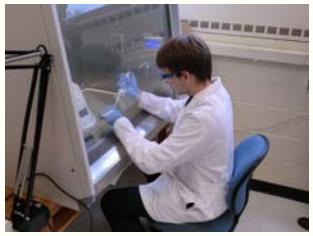

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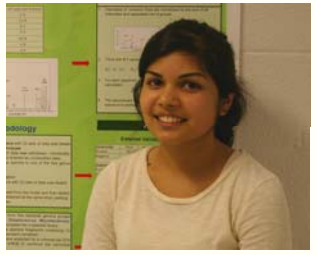
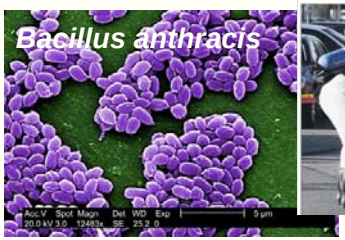


MOTIVATION: there is an urgent need right now in the military, civilian (hospital, food processing, environmental), and first responder communities for a "…rapid point-of-care diagnostic for disease-causing pathogens"

multiply drug-resistant bacteria (MDRB)

food contamination

bioterrorism &threats of bioterrorism)



emerging "super-bugs"

- ✓ lower health care costs
- ✓ improve patient outcomes
- ✓ slow the emergence of antibiotic resistance

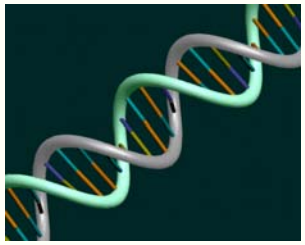
ideally this diagnostic should NOT require:

1. *a priori* knowledge of nucleic acid sequences for genetic testing
2. possession of antibodies against known bacterial antigens

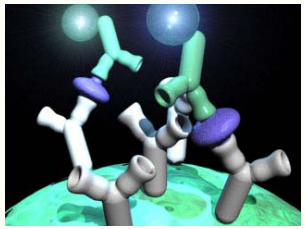
Infectious Pathogen Diagnosis

microbiological

genetic



serological



compositional

spectroscopic/spectrometric

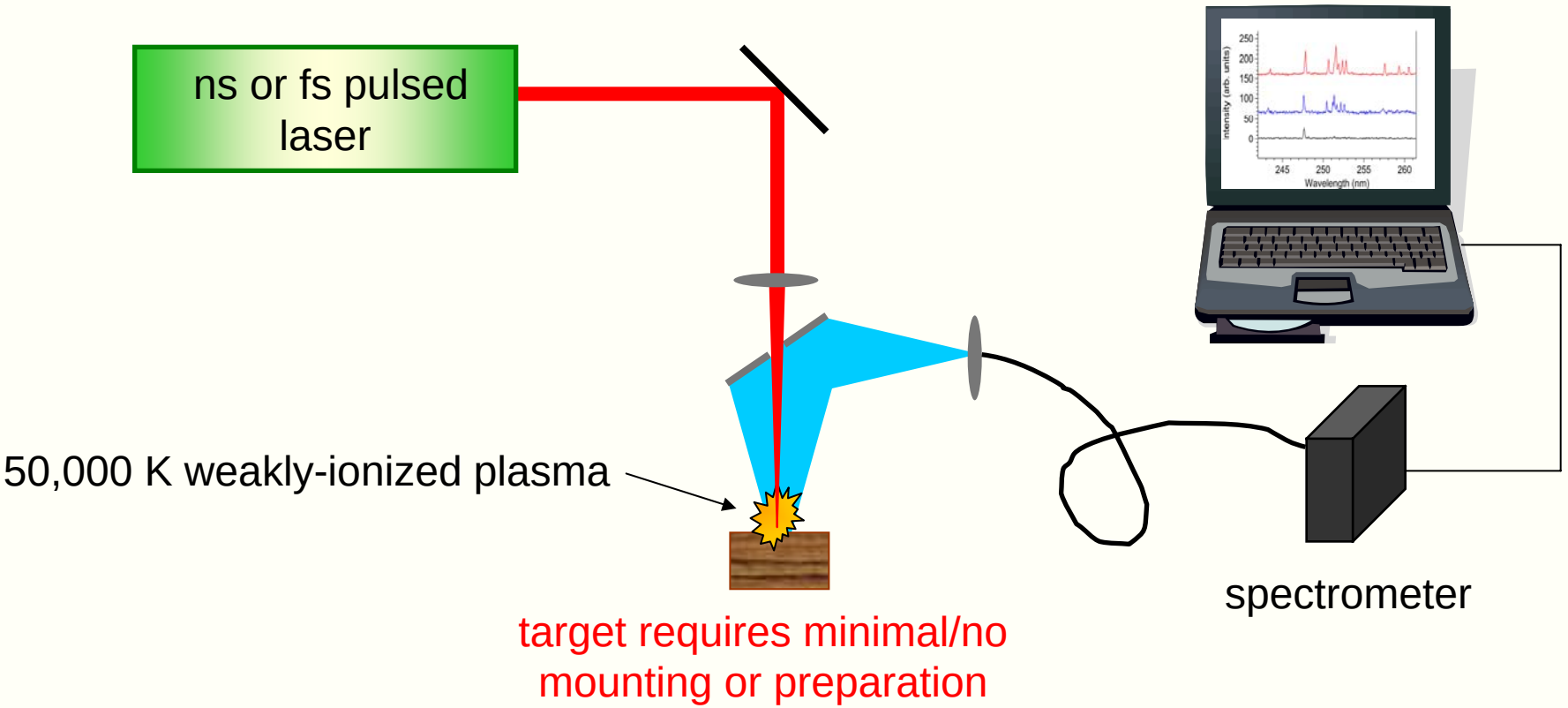
Raman spectroscopy

Laser-induced breakdown spectroscopy (LIBS)

MALDI-TOF-MS

Laser-Induced Breakdown Spectroscopy

a non-resonant laser-based atomic spectroscopy technique



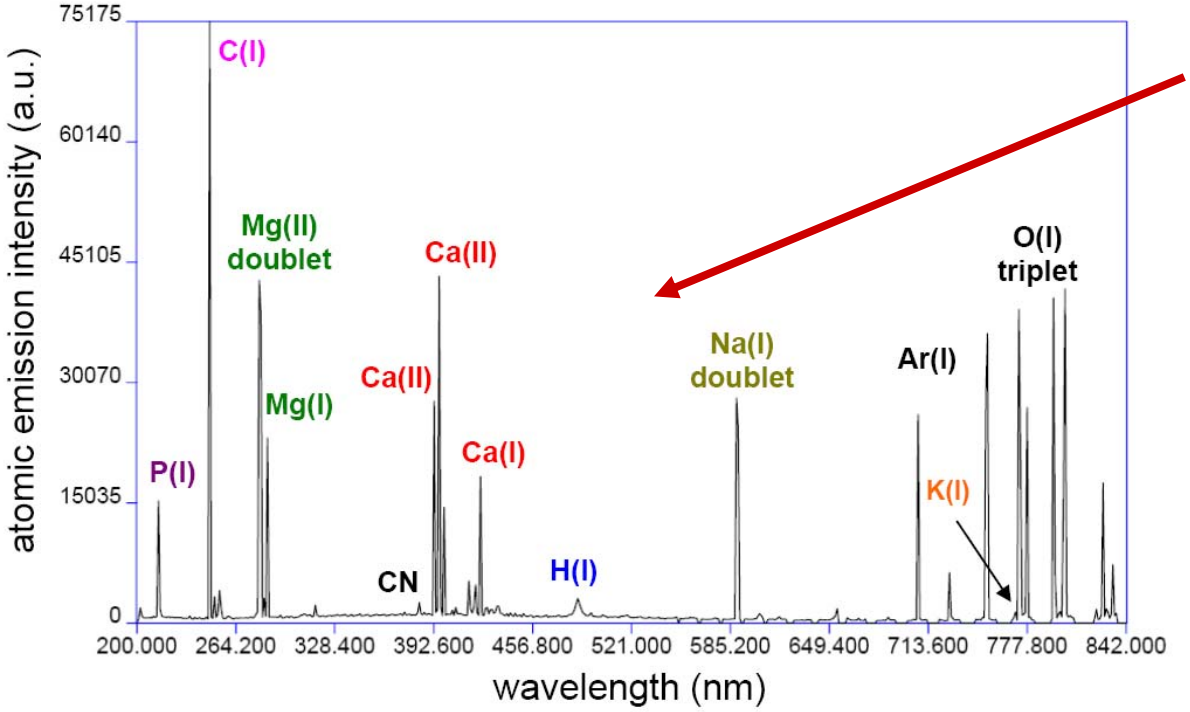
performs an elemental assay (all elements detected without bias) in under one second!

LIBS…on bacteria?

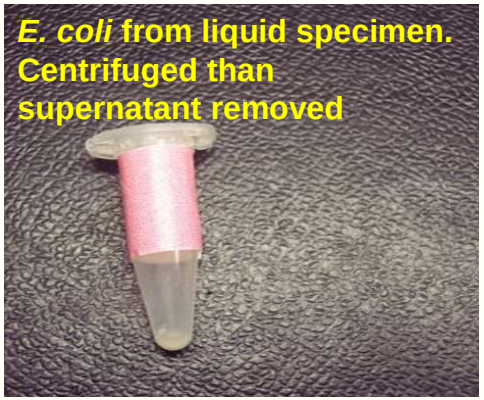
- Since 80's LIBS has been known as a fast, sensitive, and robust spectroscopic technique for rapid elemental analysis (on-line, in situ, portable)

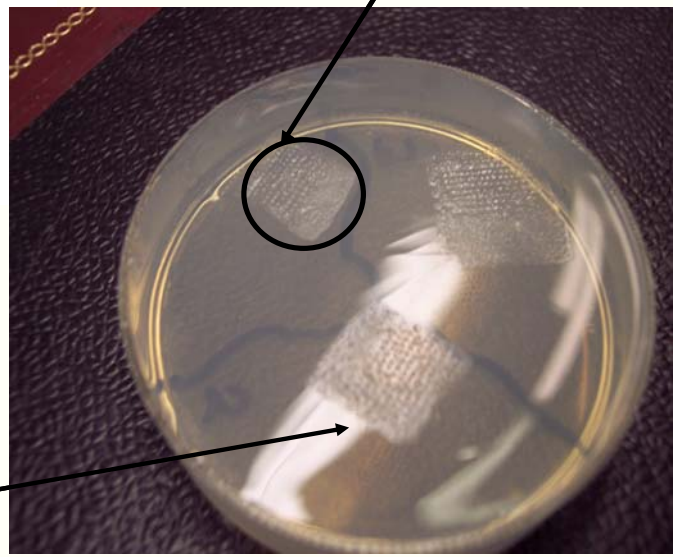
- Not enough people outside the LIBS community realize that it is currently being used for
  - molecular analysis (explosive residues, nerve agents)
  - ***analysis of complex biological specimens*** (bacteria, proteins, viruses)

A LIBS spectrum is a sensitive assay of the bacterial cell's inorganic composition



7500 cells provide a very high SNR atomic emission spectrum that is unique to each bacterial species/strain

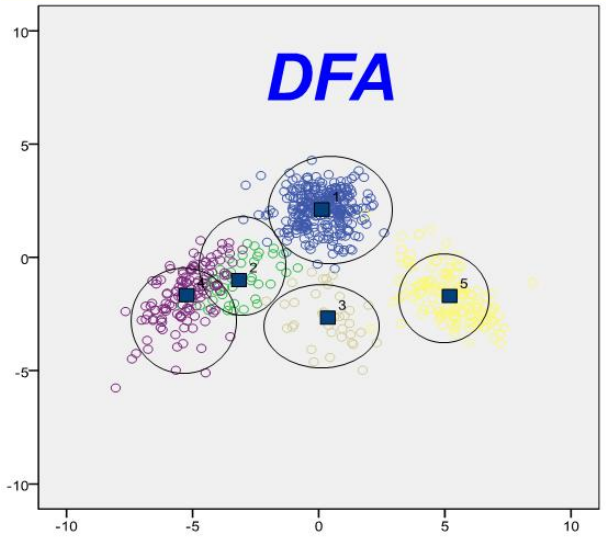


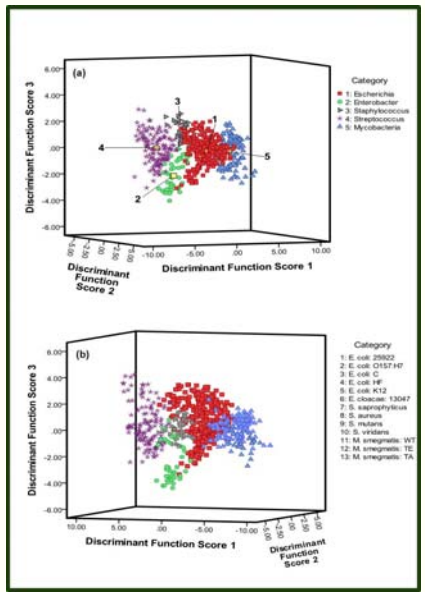


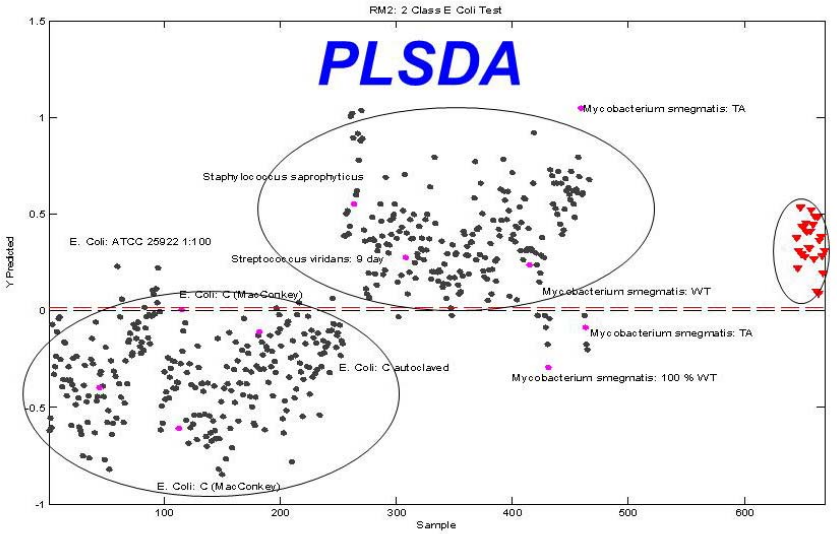
To "discriminate" one bacterial spectrum from another, a multivariate analysis ("chemometrics") is required

- Intensities of lines or ratios of intensities used as independent variables in a DFA or PLSDA
  - Express the emission intensity data in a basis set that maximizes differences between data sets
  - Build a "library" of known bacterial spectra

- Identify an unknown specimen according to which class it is assigned with the highest probability

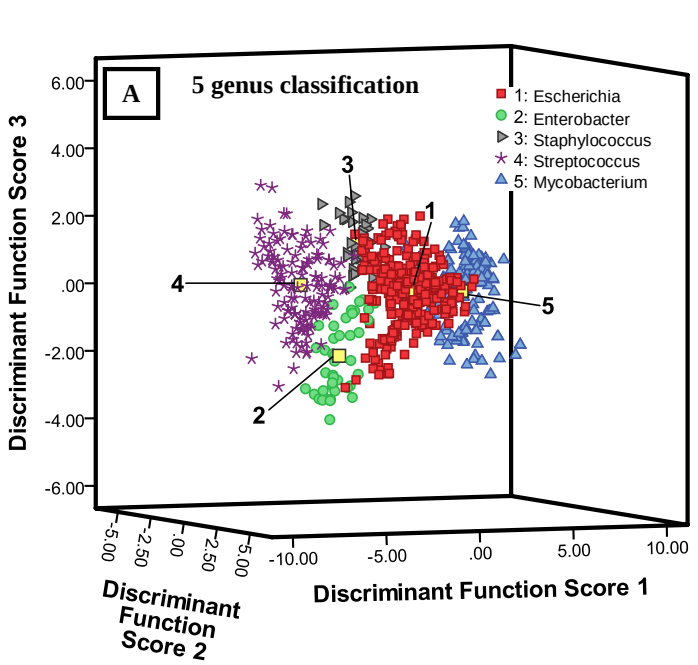






How unique is "unique"?

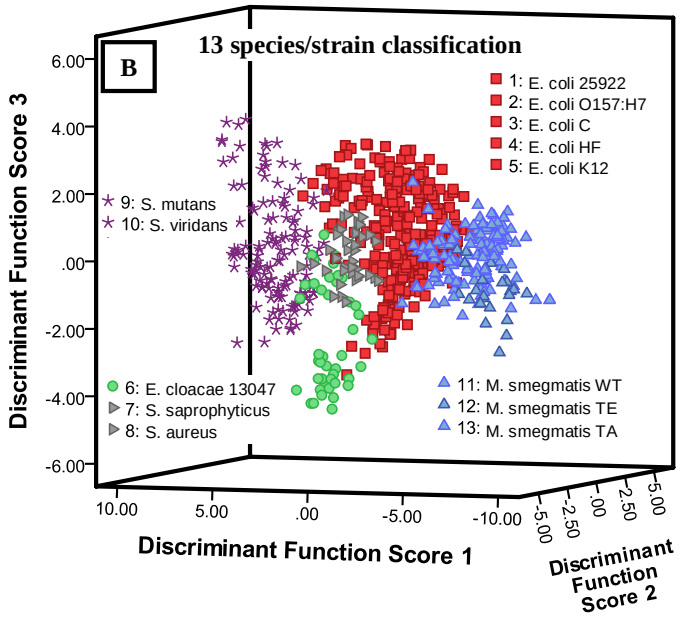
- ✓ We can identify a bacterial species, certainly its genus, with high sensitivity and specificity (confirmed by others).
- ✓ We can differentiate strains of *E. coli* (demonstrated by others in MRSA).
- ✓ Multiple multivariate techniques effective at discriminating spectra.



PLSDA			DFA		
E. COLI	True	False	*E. COLI*	True	False
Positive	95.65%	9.17%	Positive	89.63%	15.95%
Negative	90.83%	4.35%	Negative	84.05%	10.37%
STAPHYLOCOCCUS	True	False	*STAPHYLOCOCCUS*	True	False
Positive	54.05%	0.51%	Positive	86.49%	5.85%
Negative	99.49%	45.95%	Negative	94.15%	13.51%
STREPTOCOCCUS	True	False	*STREPTOCOCCUS*	True	False
Positive	95.59%	1.02%	Positive	99.26%	13.32%
Negative	98.98%	4.41%	Negative	88.68%	0.74%
MYCOBACTERIUM	True	False	*MYCOBACTERIUM*	True	False
Positive	88.31%	1.06%	Positive	96.10%	4.08%
Negative	98.94%	11.69%	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 %



Bacterial spectra are unique. Are they robust?

2007& 2011 Bacterial identification appears to be independent of the growth condition and culture medium in which the bacteria were grown.

2011 This result confirmed by Marcos-Martinez et al. on three similar growth media

2011 *Salmonella enterica* serovar Typhimuriumin identified at various concentrations in various liquids such as milk, chicken broth, and brain heart infusion.

2007-2012 The bacterial LIBS spectrum for a given species is stable and does not change with time (experiments conducted on the same *E. coli* strain over the course of multiple years).

2011 Bacterial LIBS spectra do not change with time as the bacteria age on an abiotic surface

2011 Bacterial LIBS spectra can be obtained from killed (via autoclaving) or inactivated (via UV light) specimens, and such treatment (which renders the specimen completely safe for handling) does not decrease identification specificity and does not decrease LIBS spectral intensity.

2012 Bacteria can be identified with high sensitivity and specificity when specimens are obtained from clinical samples (e.g. sterile urine containing organic and inorganic solutes) without the need to remove other compounds present in the sample.

2012 Live pathogenic *Bacillus anthracis* Sterne strain and *Francisella tularensis* can be differentiated regardless of mounting protocol (as lawn and/or colonies on agar, dilutions on agar, and dilutions on glass slides.)

2011 2012 Bacteria in mixed samples are identifiable. The dominant or majority bacterial component of a two-component bacterial mixture is reliably identified provided it comprises 70% of the mixture or more. Trace mixture or contamination is insignificant.

Where are we going next?

(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

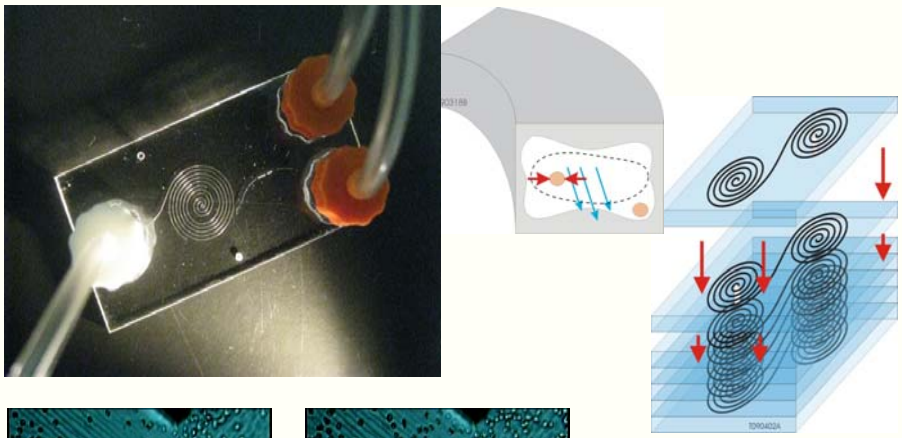
(2) Strain classification (particularly antibiotic-resistant pathogen strains such as MRSA)

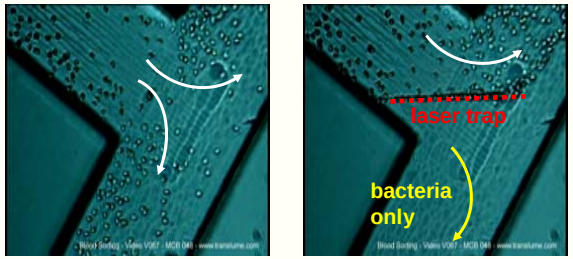
These two applications alone (MRSA infections and UTI's) are responsible for over $2 billion of medical costs worldwide every year.

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*.

Where are we going next?

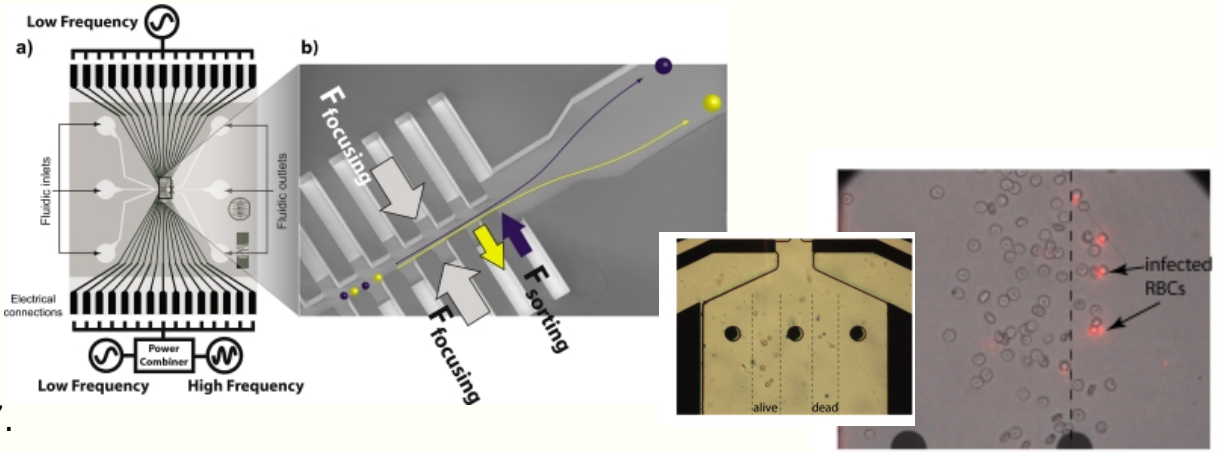
Microfluidic separation/concentration





Centrifugation/filtration

Dielectrophoresis



A miniaturized continuous dielectrophoretic cell sorter and its applications

Ana Valero, Thomas Braschler, Nicolas Demierre, and Philippe Renaud

Biomicrofluidics. 2010 June; 4(2): 022807.

Long-term goals to improve the health of Canadians.

(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 and prototypes developed.

Much remains to be done…

…but all tests to date have proven the possibility of using LIBS for a rapid pathogen diagnostic, as well as numerous other biomedical applications.

Work continues, with generous help from:

- University of Windsor

University of Windsor
thinking forward

- NSERC Discovery Grant

Natural Sciences and Engineering Research Council of Canada
Conseil de recherches en sciences naturelles et en génie du Canada

- CFI-LOF grant

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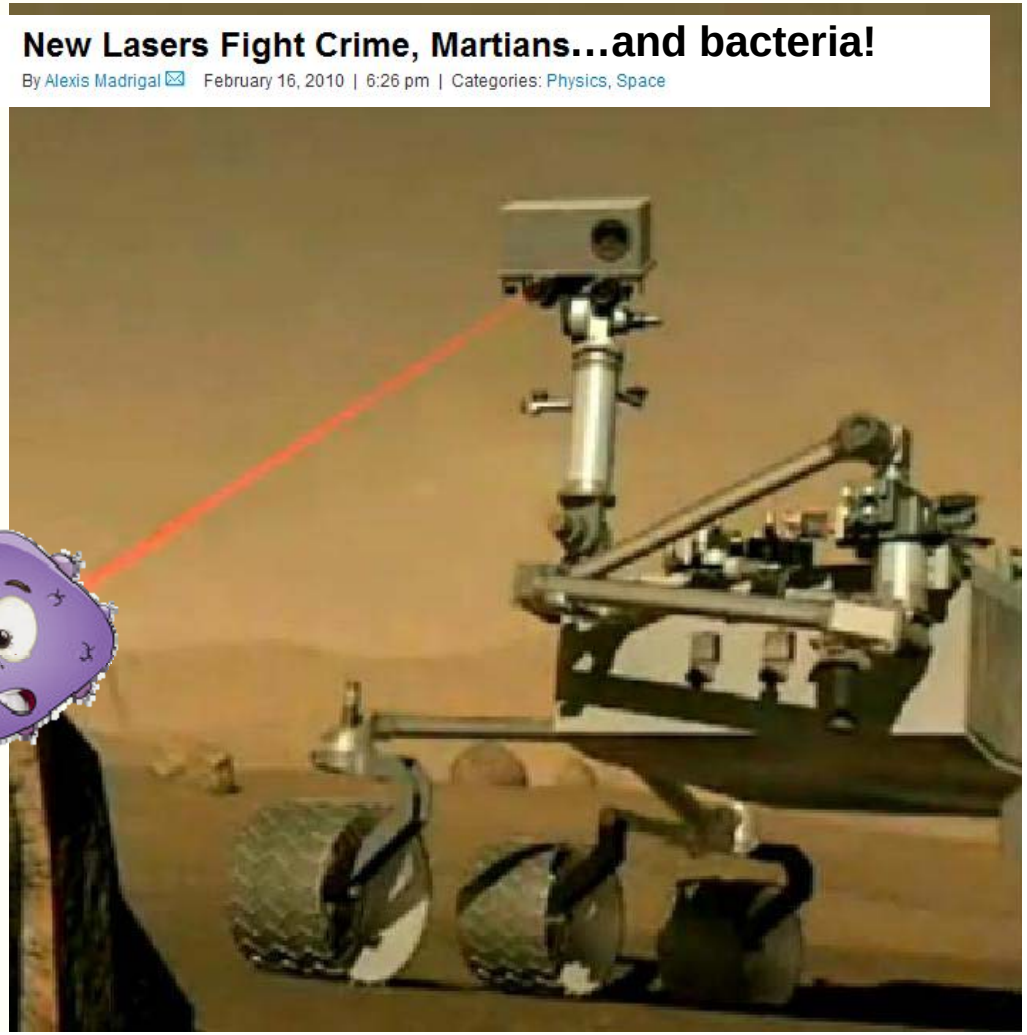

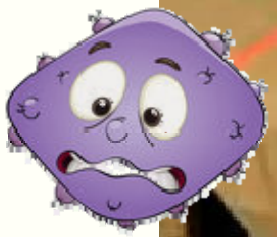

The Mars Science Laboratory “Curiosity” uses the ChemCam LIBS package to ablate rocks looking for signs of habitable environments.