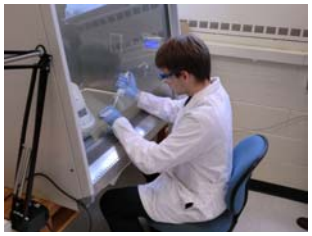
Laser-induced breakdown spectroscopy (LIBS): a new paradigm for rapid pathogen identification

Steven J. Rehse

Qassem Mohaidat *WSU, Dept. of Physics and Astronomy*
Khozima Hamasha *WSU, Dept. of Physics and Astronomy*

Sunil Palchaudhuri *WSU, Dept. of Immunology and Microbiology*
Hossein Salimnia *WSU, Dept. of Pathology / Detroit Medical Center*
Choong-Min Kang *WSU, Dept. of Biological Sciences*

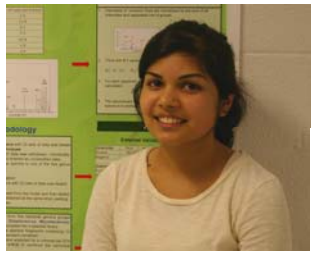
Laser-induced breakdown spectroscopy (LIBS): a new paradigm for rapid pathogen identification


Khadijia Sheikh, Russell Putnam, Andrew Daabous, Ryan Woodman, Daniel Trojiand, Eric Lessard, Derek Gillies

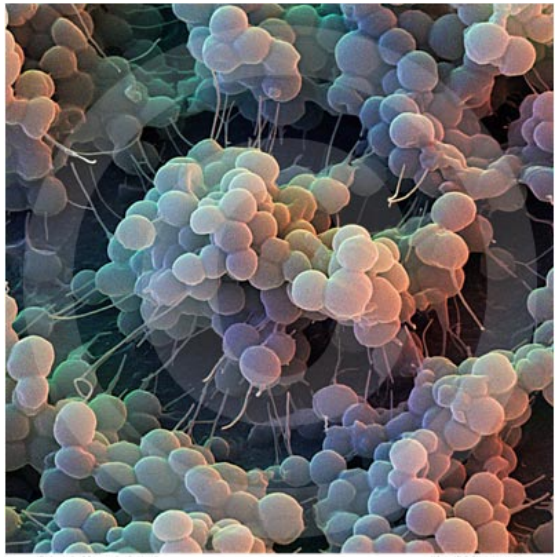
University of Windsor, Department of Physics





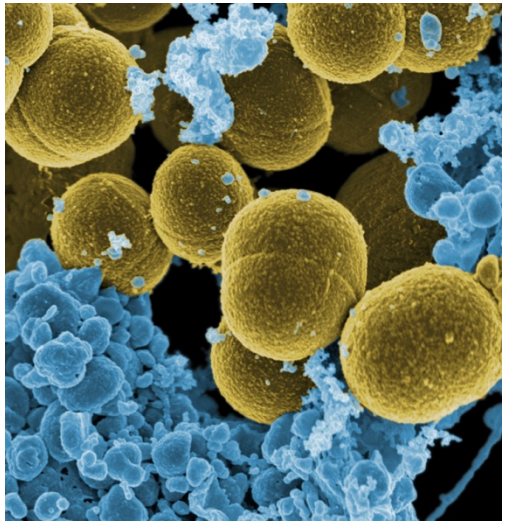

Andrzej W. Miziolek *US Army Research Laboratory, APG, MD*

Leslie M. Collins *Duke University, Durham, NC*
Peter A. Torrione *Duke University, Durham, NC*



Staph. epidermidis

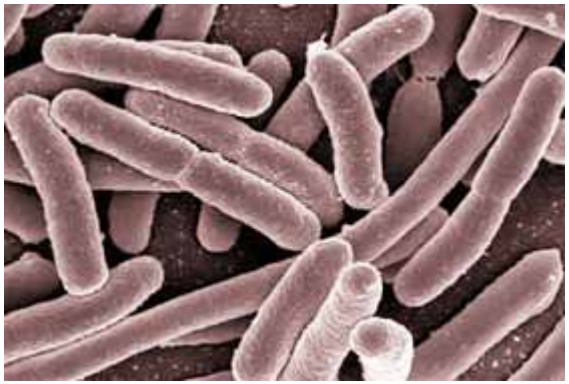
Staph. aureus



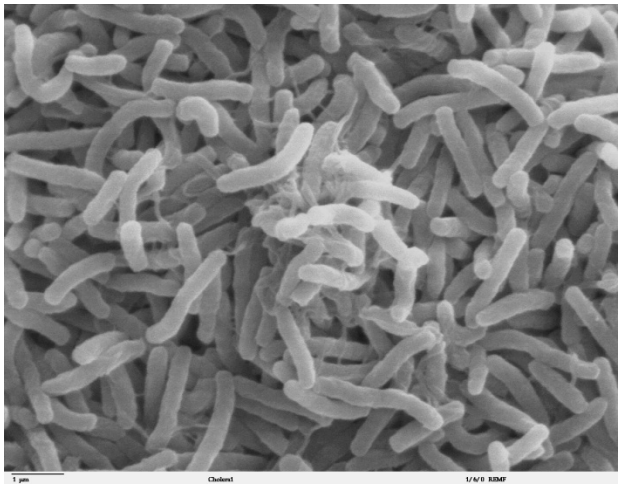
bacteria are ubiquitous

10x more prokaryotic cells in your body than eukaryotic cells

E. coli



V. cholerae



updated 9:31 a.m. EST, Mon March 2, 2009

Antibiotic-resistant infections among children on the rise

CNNhealth.com

updated 12:52 p.m. EDT, Sun August 24, 2008

Canada links Toronto plant to deadly listeriosis outbreak

USA TODAY Home News Travel Money Sports Life

Nation Inside News

E. coli kills Idaho toddler; spinach pla probed

Updated 10/5/2006 8:57 PM ET

E-mail | Save | F

December 8, 2003

Staph Infection Kills Football Player

By Norm Jones, Newswatch 16, Scranton, PA

Denver News

CU's Nobel Prize Winner Loses Arm To Flesh-Eating Bacteria

Eric Cornell Remains In Critical Condition

The New York Times

eanut Product Recall Grows in Salmonella Scare

GARDINER HARRIS

olished: January 28, 2009

CBCnews

Home World Canada Politics Health Arts & Entertainment Technology & Science Money Cons

REPUBLISH | EMAIL | PRINT | Text Size: S M L XL | REPORT TYPO | SEND YOUR FEEDBACK | SHARE

New superbugs emerge in U.K., Asia

Canadian cases reported in Vancouver, Alberta

Last Updated: Sunday, August 15, 2010 | 10:18 PM ET Comments 447

USA TODAY Home News Travel Money Sports

News » Health & Behavior ▪ Medical Resources ▪ Health Information

CDC: 756 ill from salmonella-tainted tomatoes

Windsor Star Digital
THE WINDSOR STAR DIGITAL
THE WINDSOR STAR
THE WINDSOR STAR

NEWS / Police And Fire

Suspicious powder at National Bank not dangerous, police say

Firefighters and hazardous material specialists gather on Pitt Street West in response to a report of a suspicious white powder at the Canada Post building on Ouellette Avenue in Windsor, Ont. on April 18, 2012. (Nick Brancaccio / The Windsor Star)

A hazardo
Windsor
fire and ambulance personnel des
inside a pencil case that had beer
after 4 p.m., two firefighters donnec
workers sifted through the found ob
origin or makeup of the powder. It has been taken to a laboratory in Etobicoke for testing.

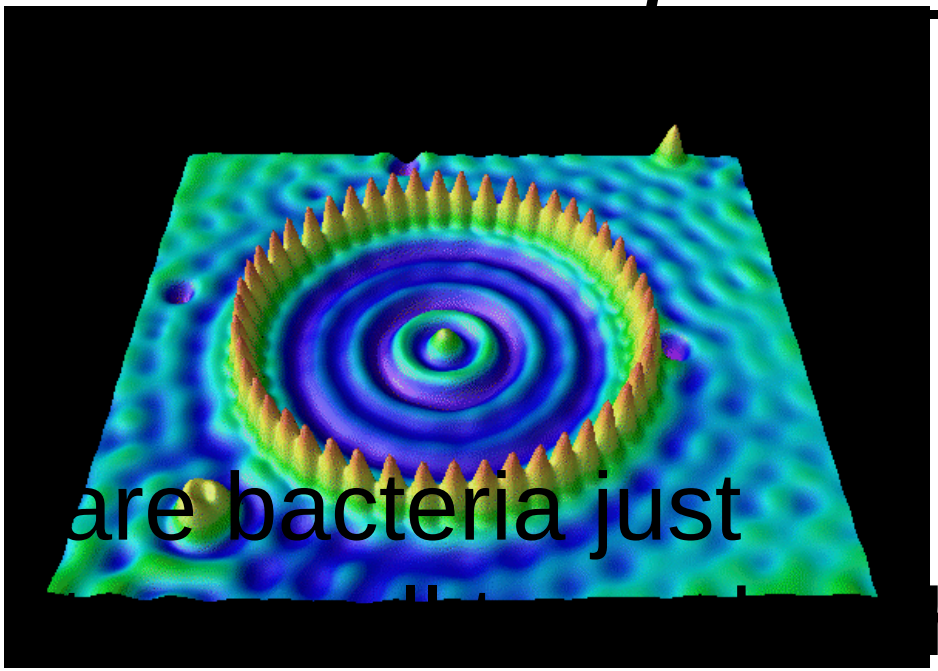
So why?

"It is well-accepted that the microbiological expertise and cost required to perform these identifications preclude their common use as a screening mechanism to prevent human infection."[1]

[1]Tarr, P.I. 1995. *Escherichia coli* O157:H7: clinical, diagnostic, and epidemiological aspects of human infection. Clin. Infect. Dis. 20, 1-8.

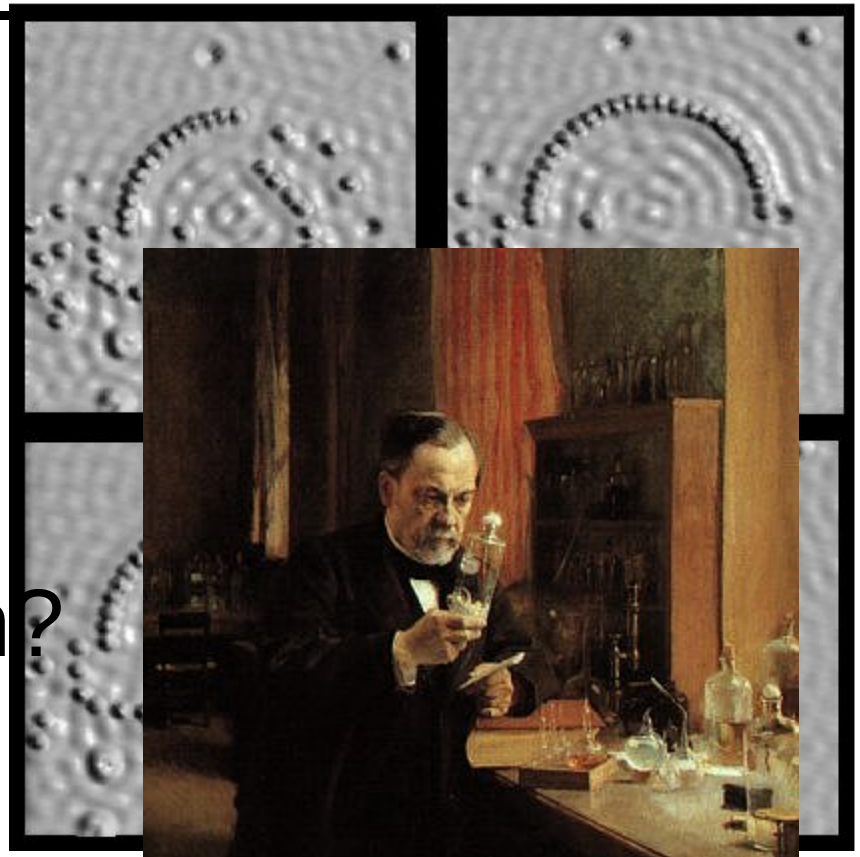
“Too small?”

What’s the problem?

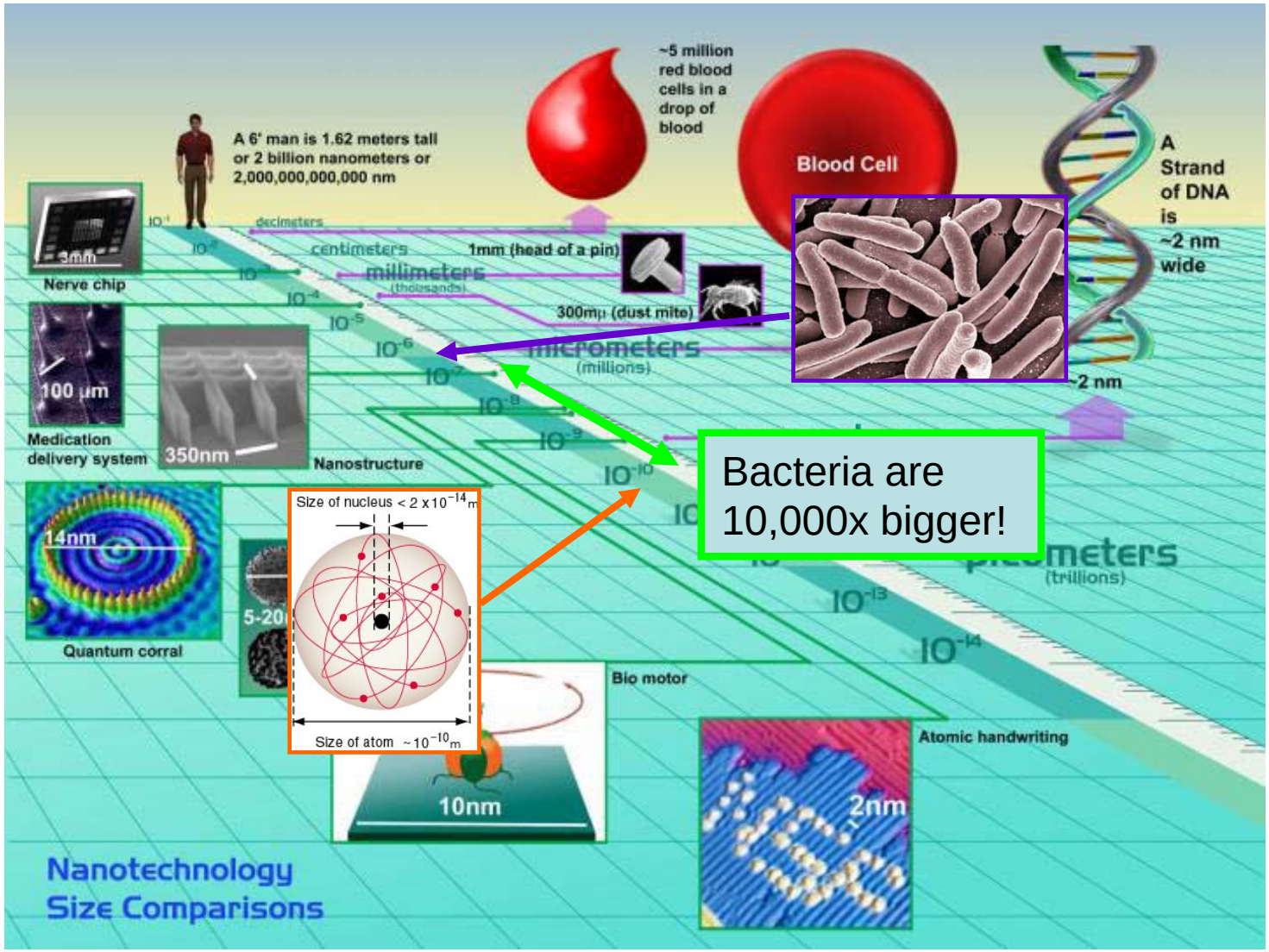


"Quantum Corral"

Scanning Tunneling Microscope image of individual iron atoms arranged intentionally on a copper surface in a circular ring, exposing quantum electron waves



AFM i eing



From “Nanopedia” at Case Western University

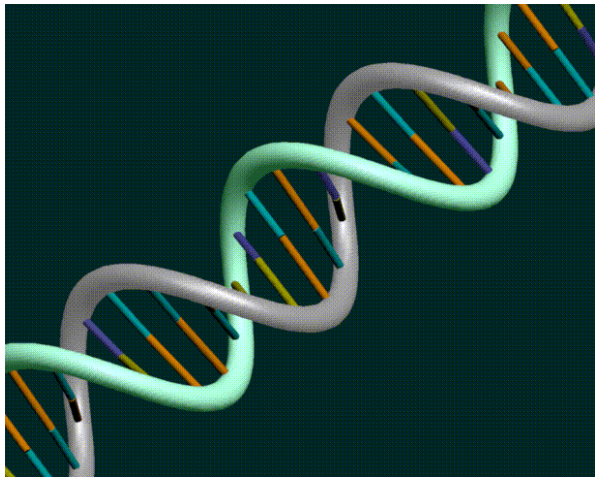
If it's not the size, it must be our methods

How do we identify bacteria?

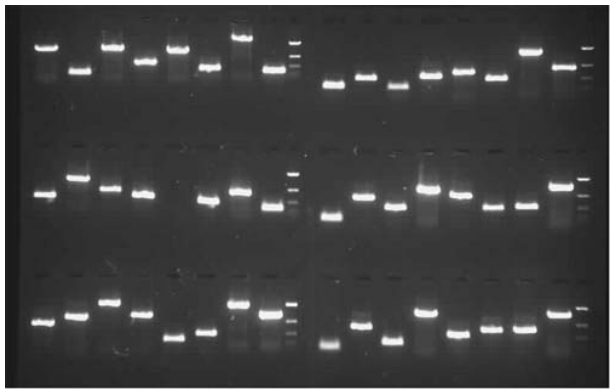
4 ways

- ***genetic***
- ***serological (antigenic)***
- ***microbiological (phenological)***
- ***compositional***

genetic



- PCR (polymerase chain reaction)
- (random primed) RAPID-PCR
- FISH (fluorescence *in situ* hybridization)



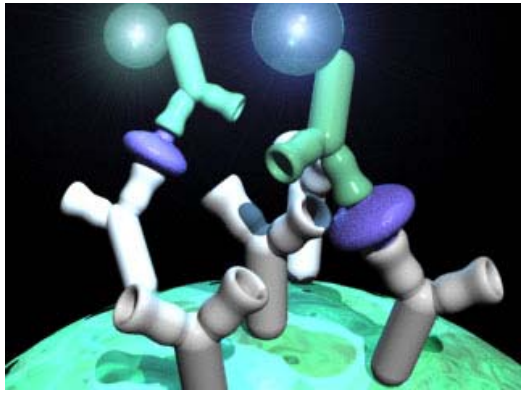
requires

- *a priori* knowledge of genetic sequence (16s RNA gene is conserved in most)

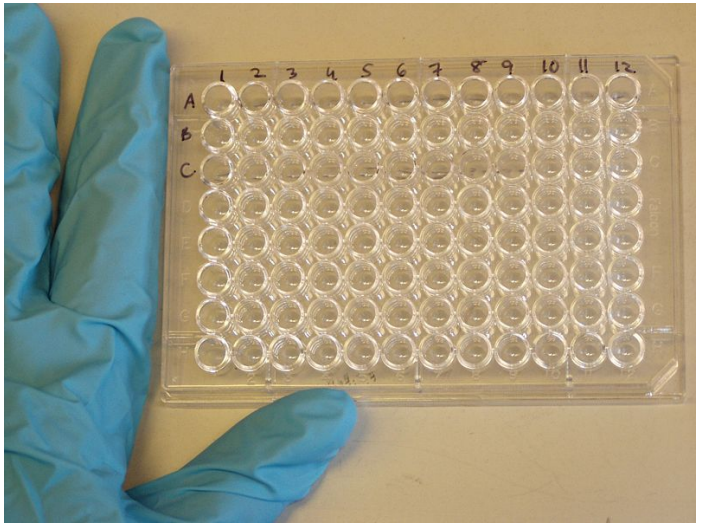
drawbacks

- amplification time (multiple generations needed)
- nonspecific reactivity
- still need to do gel electrophoresis
- very contamination sensitive

serological



- immunoassays
- microwell devices
- ELISA (enzyme-linked immunosorbent assay)
- fluorescently labeled antibody techniques
- MEMS



requires

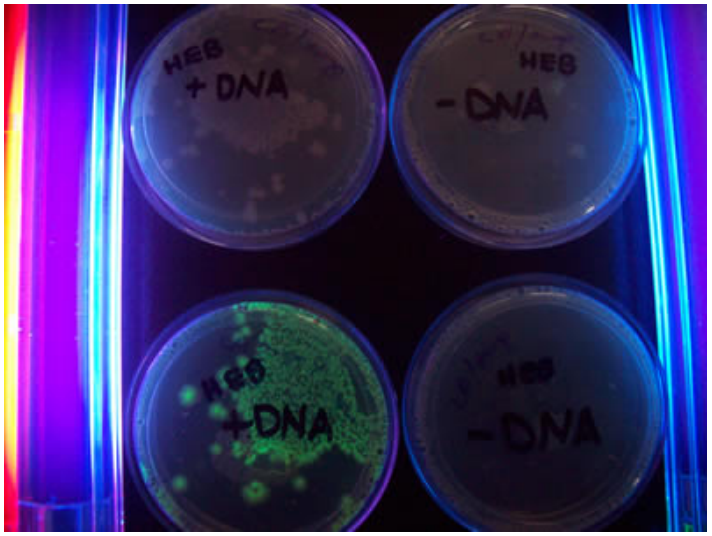
- *a priori* knowledge of serology (surface antigens)

drawbacks

- any mutation (common) undetectable
- antibodies are not stable (shelf-life)
- consumables
- binding affinities may be low

microbiological

- culturing and colony counting
- phenotyping
- sensitivity to immunochemicals
- Gram staining



requires

- time
- expertise
- LOTS of supplies
- *a priori* clinical knowledge (case-history)

drawbacks

- slow/labor intensive
- requires experts

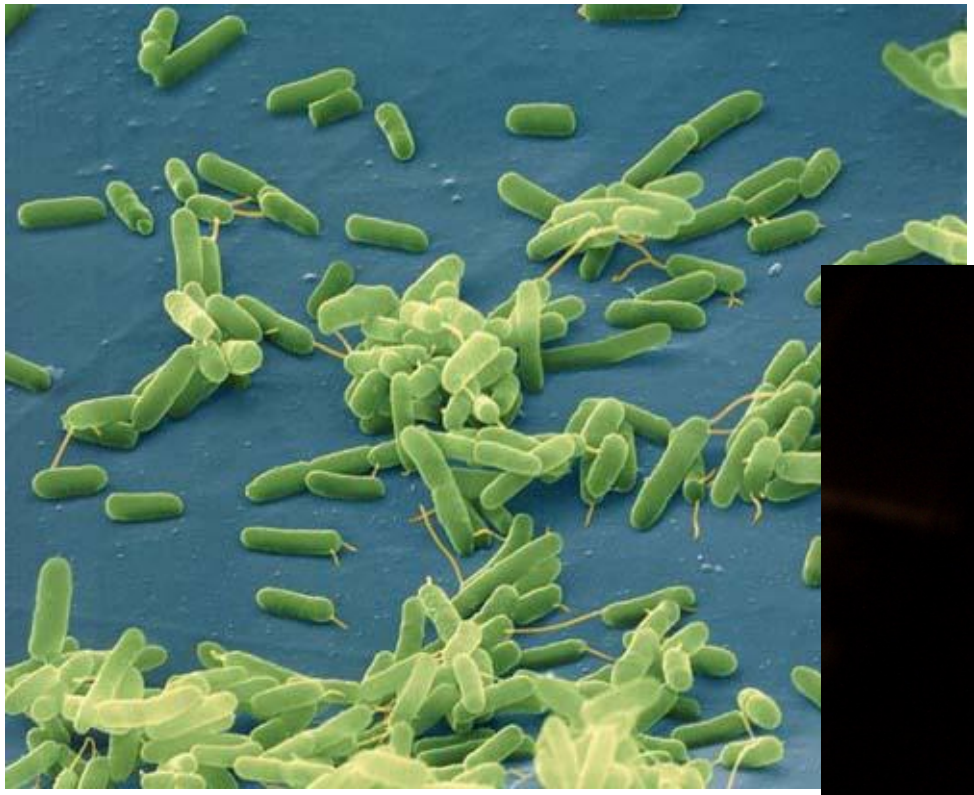
compositional

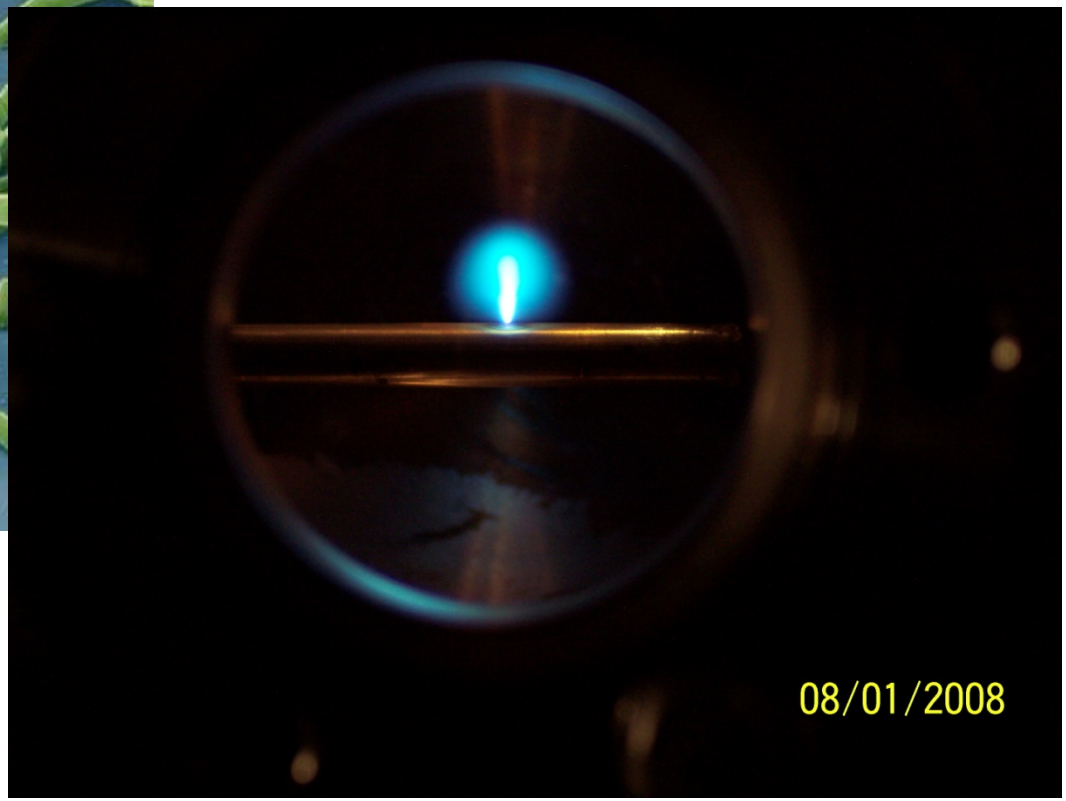
- Mass-spectrometry (MALDI-TOF-MS): fragments
- Raman spectroscopy: molecules
- Laser-induced breakdown spectroscopy (LIBS): atoms

requires

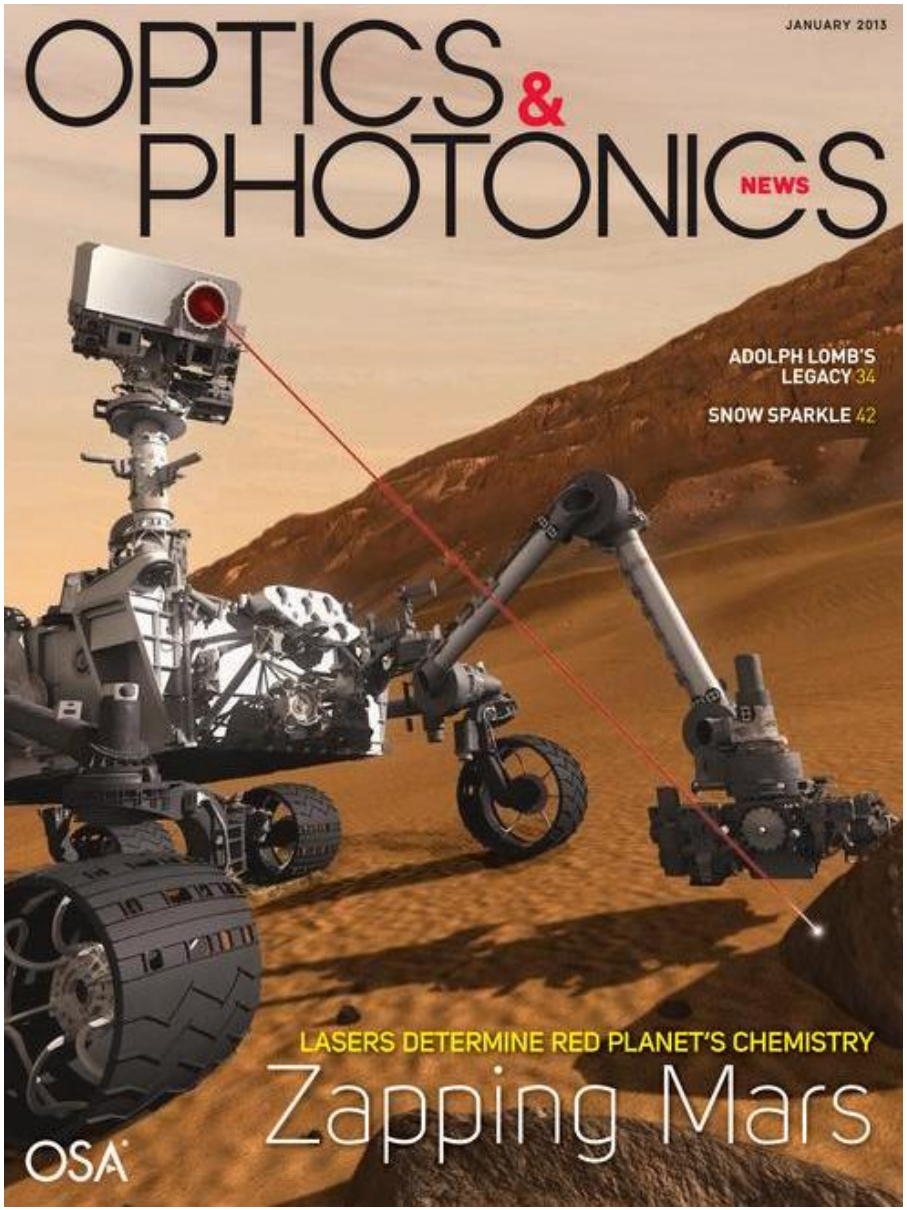
- no *a priori* knowledge of serology (surface antigens)
- no *a priori* knowledge of genetic sequence
- no consumables (hopefully)
- no expertise (objective diagnosis)

A Brief Introduction to Laser-Induced Breakdown Spectroscopy (LIBS)





LIBS zapping Martians…



WIRED SUBSCRIBE » SECTIONS » BLOGS » REVIEWS » VIDEO » HOW-TOS »

Sign In | RSS Feeds

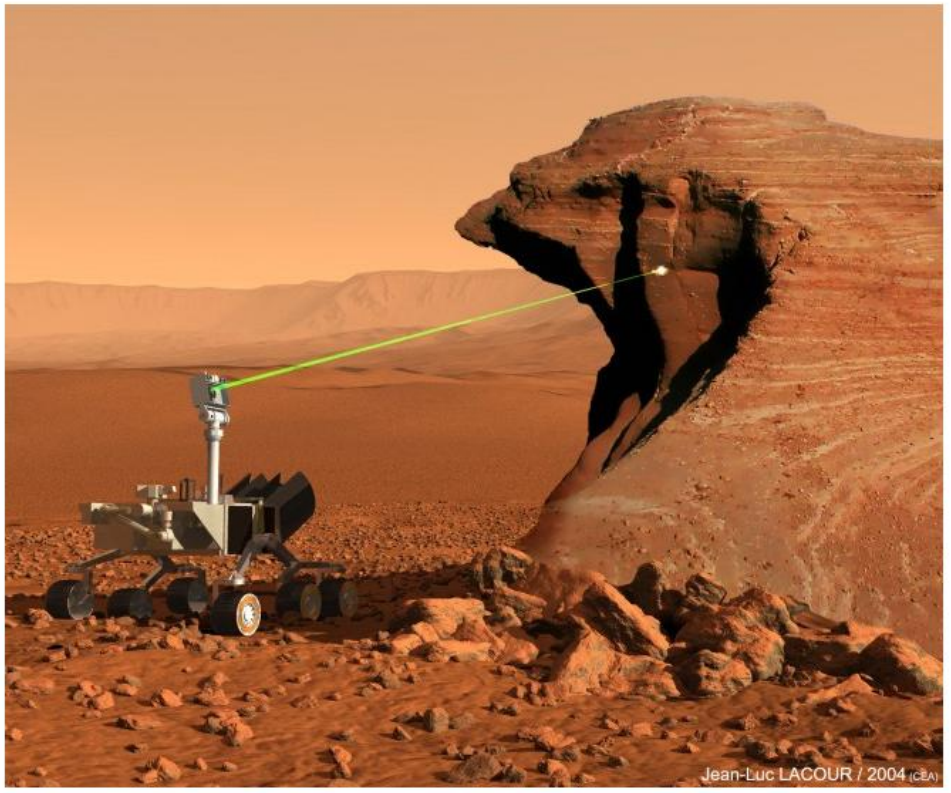
WIRED SCIENCE

NEWS FOR YOUR NEURONS

PREVIOUS POST NEXT POST

New Lasers Fight Crime, Martians

By Alexis Madrigal | February 16, 2010 | 6:26 pm | Categories: Physics, Space



A new technique that uses a laser to vaporize materials like rocks and steel to analyze their chemical composition is finding new applications from Mars to forensics.

…and Microbes???

MP-LIBS A full laboratory High-Resolution Broadband LIBS system in a portable backpack

Backpack contains broadband high-resolution spectrometer, laser power supply, computer, and battery

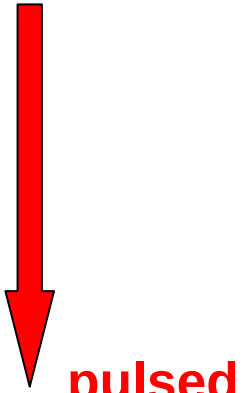
Head's-up display

Hand-held probe contains laser, joystick for control, and focus optics

Microplasma/ LIBS Event

courtesy of Ocean Optics.

1) laser interaction with the target

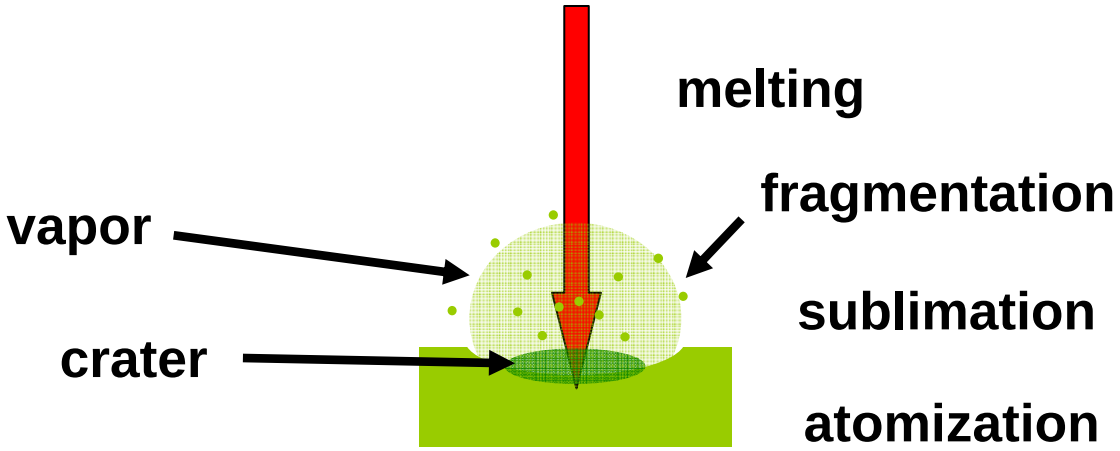


pulsed laser

absorption of laser energy

- initiated by absorption of energy by the target from a pulsed radiation field.
- pulse durations are on the order of nanoseconds, but LIBS has been performed with pico- and femto-second laser pulses.

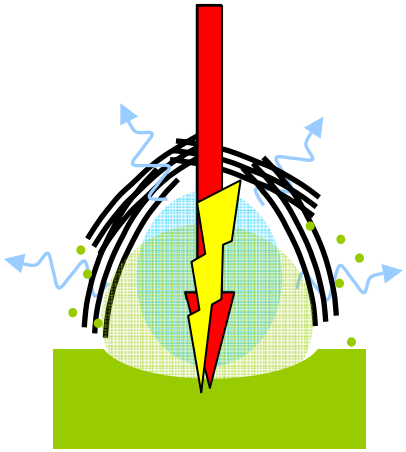
2) removal of samples mass (ablation)



- absorbed energy is rapidly converted into heating, resulting in vaporization of the sample (ablation) when the temperature reaches the boiling point of the material.

- removal of particulate matter from the surface leads to the formation of a vapor above the surface.

3) plasma formation (breakdown)



absorption of the laser radiation by the vapor

continuum emission

electrical breakdown and plasma formation

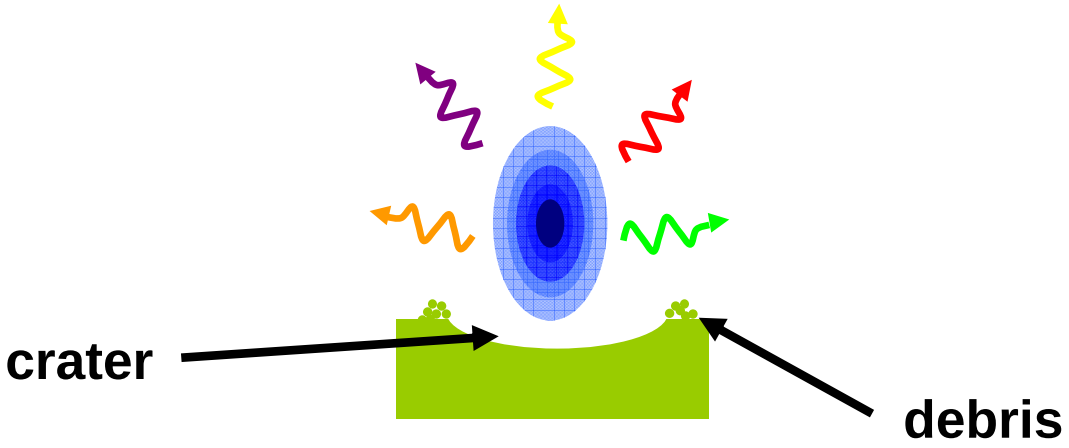
shock wave

bremsstrahlung

- The laser pulse continues to illuminate the vapor plume.
- The vapor condenses into sub-micrometer droplets that lead to absorption and scattering of the laser beam, inducing strong heating, ionization, and plasma formation.

4) expansion and element specific emission (atomic or ionic)

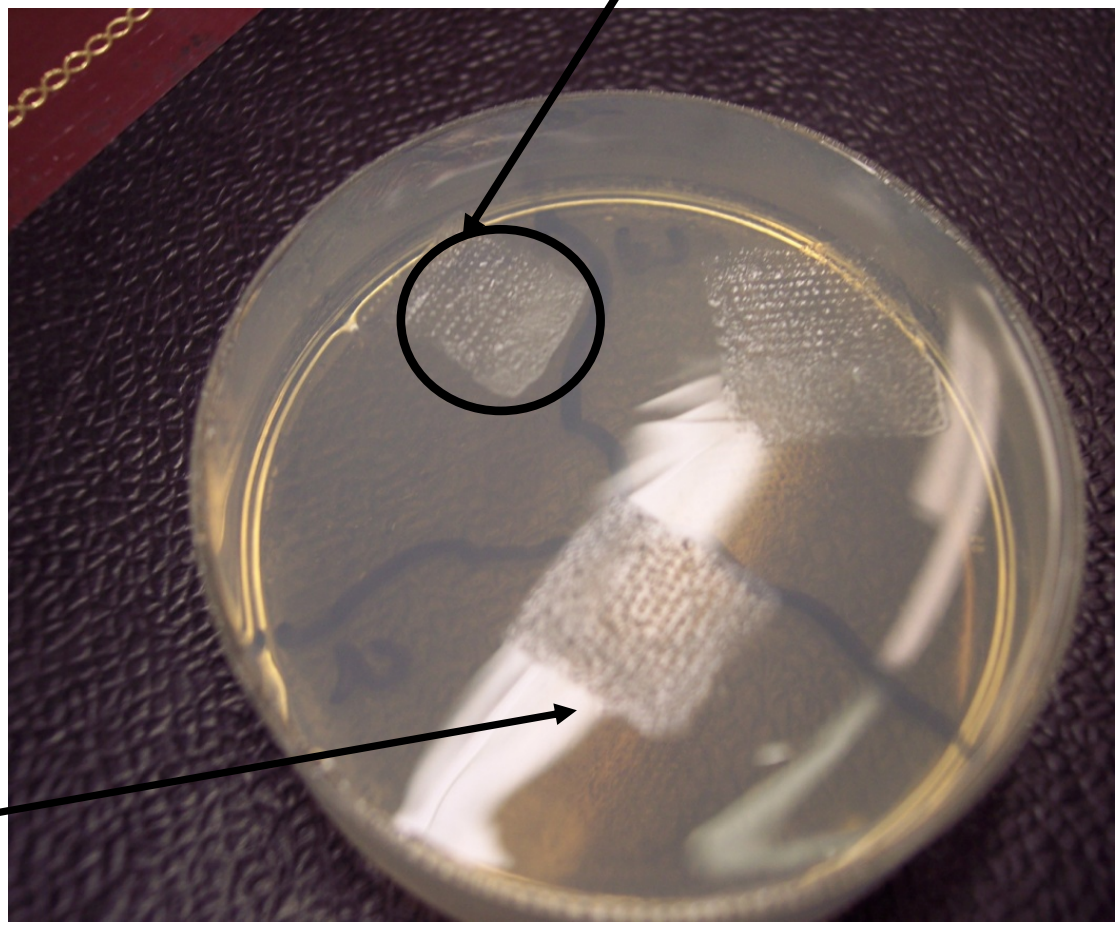
spontaneous emission as atoms/ions decay to ground state



- The dynamical evolution of the plasma plume is then characterized by a fast expansion and subsequent cooling.
- Approximately 1 microsecond after the ablation pulse, spectroscopically narrow atomic/ionic emissions may be identified in the spectrum.

how we do it…

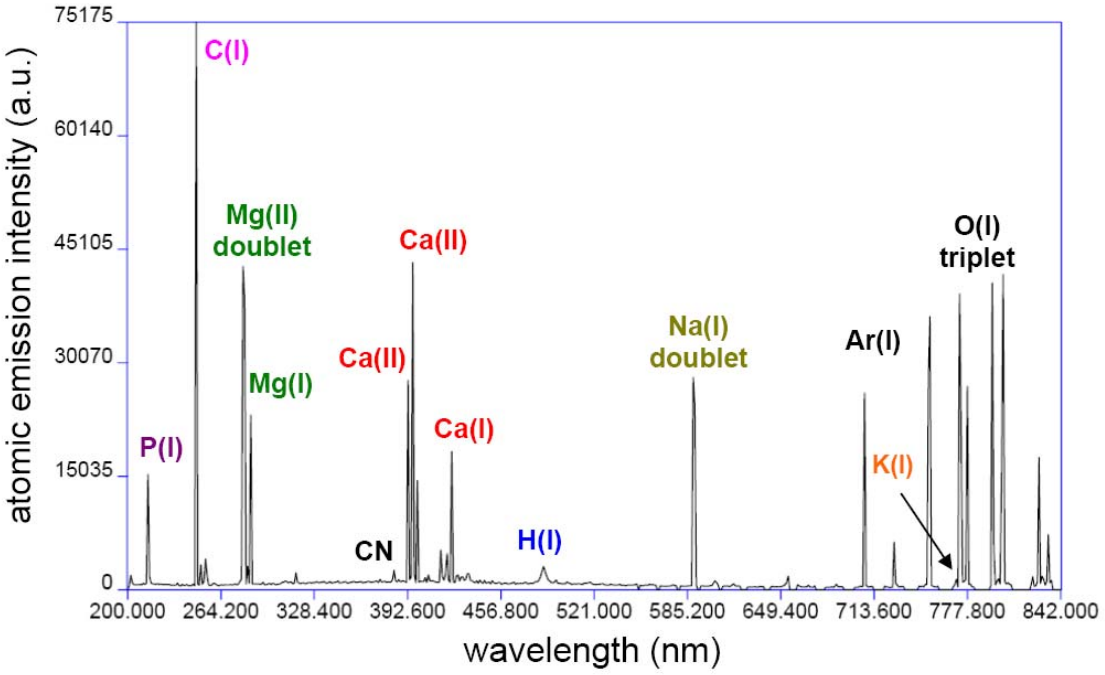
10 microliter



about 500-1500
bacteria per
sampling location

bacterial composition

Ratios of elements create a unique "spectral fingerprint" for each bacteria.

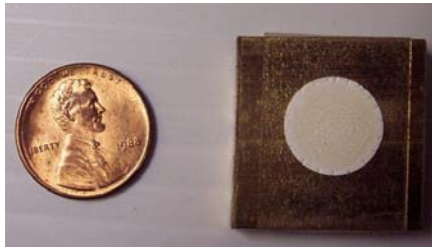


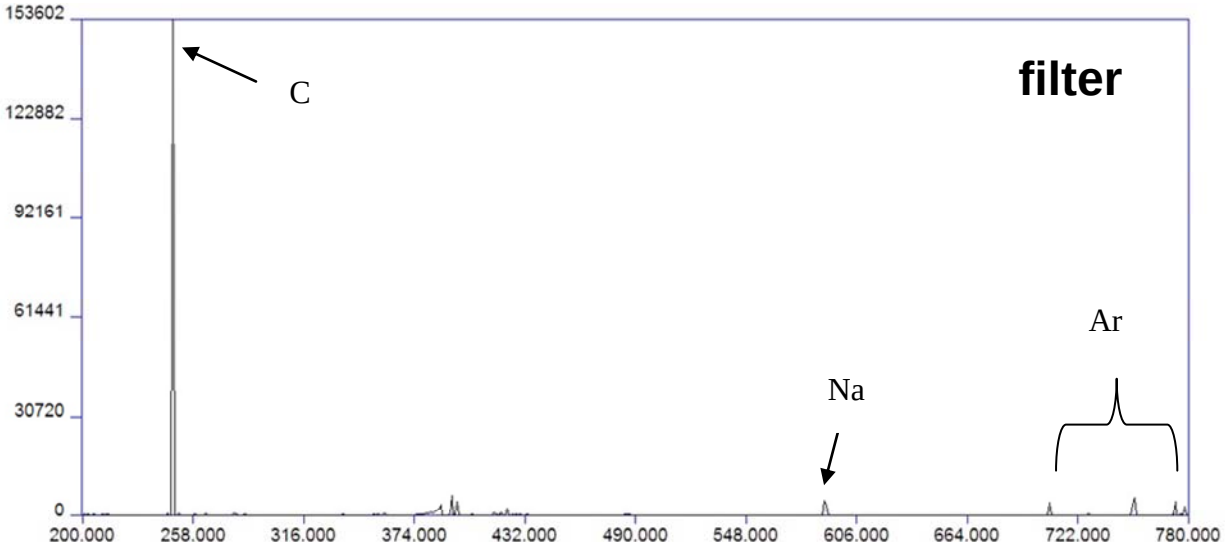
LIBS-based pathogen identification is inorganic element based (at this point)

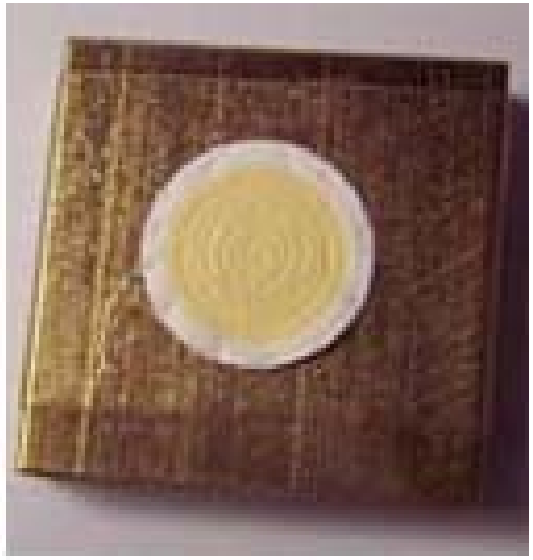
from "*The Bacteria: A Treatise on Structure and Function*" I.C. Gunsalus and R.Y. Stanier, eds

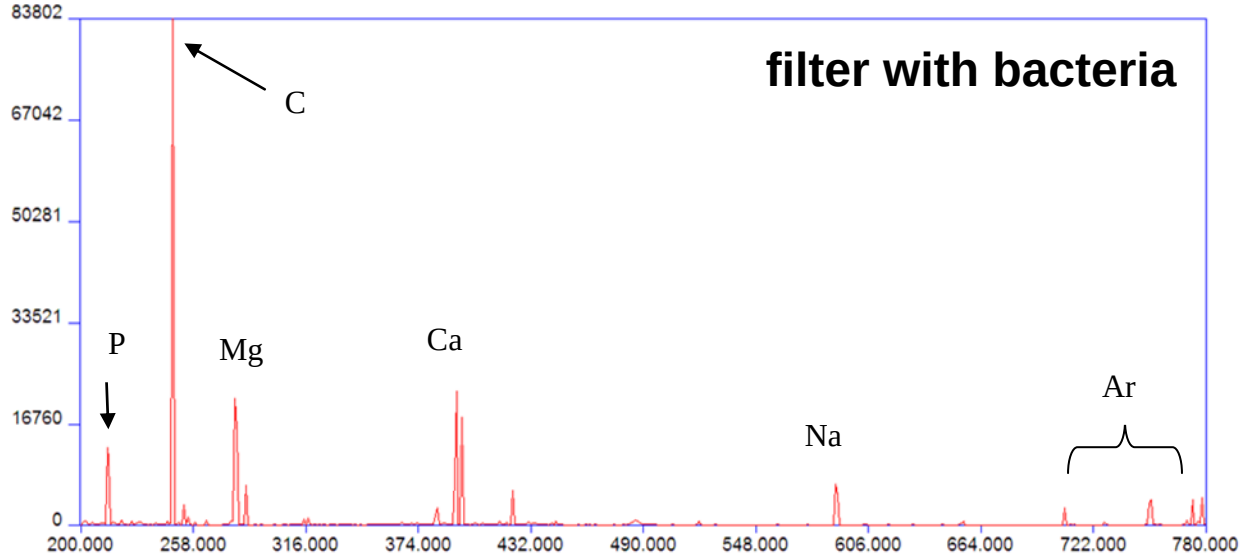
Element	% of fixed salt fraction
Sodium	2.6
Potassium	12.9
Calcium	9.1
Magnesium	5.9
Phosphorus	45.8
Sulfur	1.8
Iron	3.4

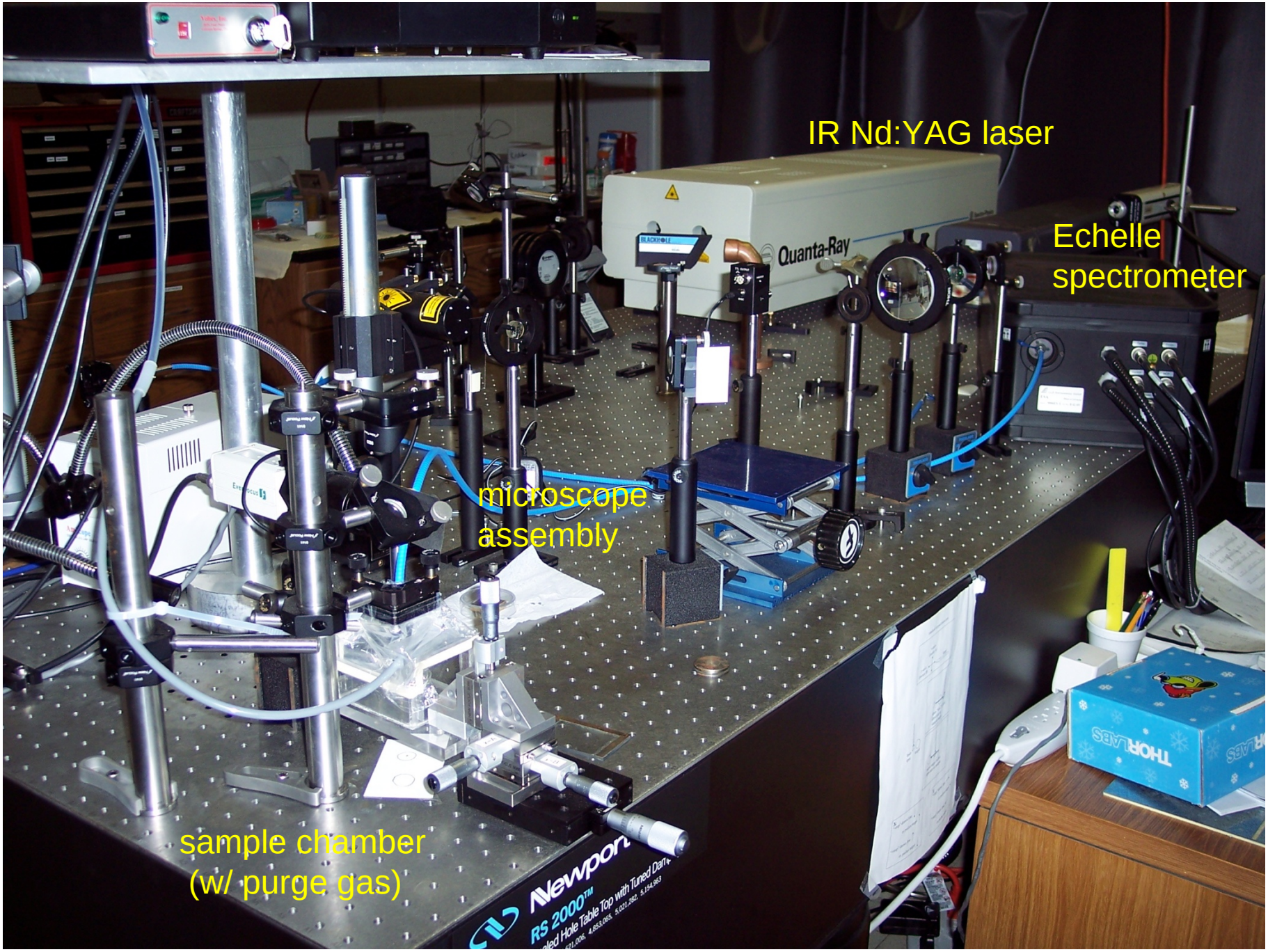
Cellulose Filter









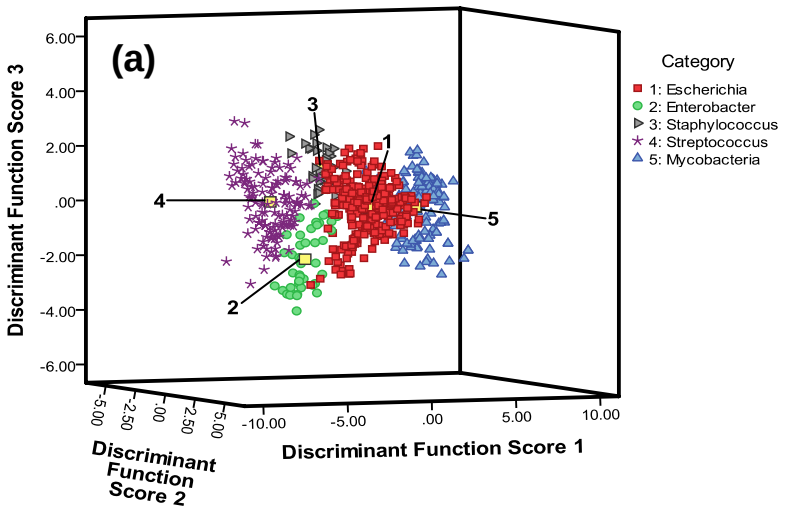

IR Nd:YAG laser
Quanta-Ray
Echelle
spectrometer
microscope
assembly
sample chamber
(w/ purge gas)
Newport
RS 2000™
THORLABS

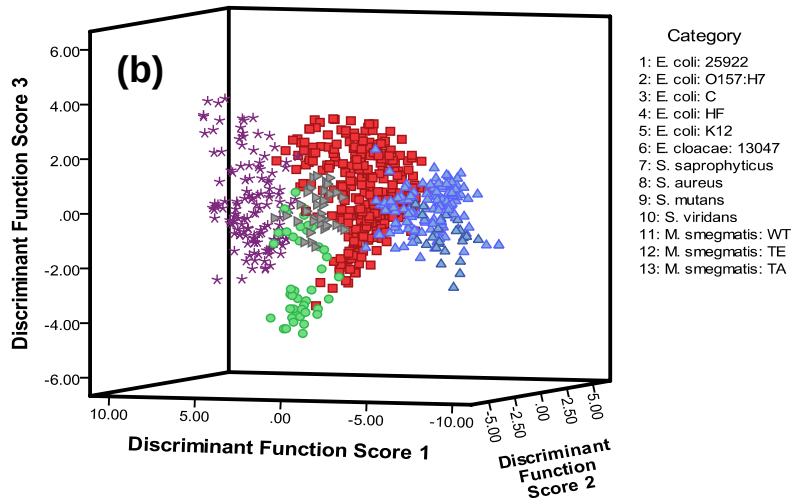
chemometrics used

- To discriminate highly similar LIBS spectra – sophisticated multivariate analyses - "*chemometrics*" - must be utilized
- The intensities of 13 atomic emission lines are used as independent variables.
- Here, LIBS spectra from 13 different bacterial types were input into the DFA – no relationships between the bacteria were provided.
- We plot the results in a 3D space (but the groupings exist in a 12D space).

discriminant function analysis: DFA

partial least squares-discriminant analysis: PLS-DA





Results: We have already demonstrated…

- LIBS spectral fingerprint is a *sensitive* and *specific* (high rates of true positives, low rates of false positives) test to identify an unknown bacterial specimen or to differentiate between possible identifications
- This spectral fingerprint is *robust* and *reliable*, and exists through time (multiple tests spanning years on same strains of bacteria)
- In addition…

8 publications in Applied Physics Letters, Journal of Applied Physics, Applied Optics, Applied Spectroscopy, Spectrochimica Acta B, and others – confirmed by multiple other groups

Results: We have already demonstrated…

LIBS 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 (or inactivated by UV light)
- obtainable even when other types of bacteria or contaminants are present (mixed samples)
- obtainable from urine specimens
- capable of strain discrimination
- obtainable from about 500 bacteria

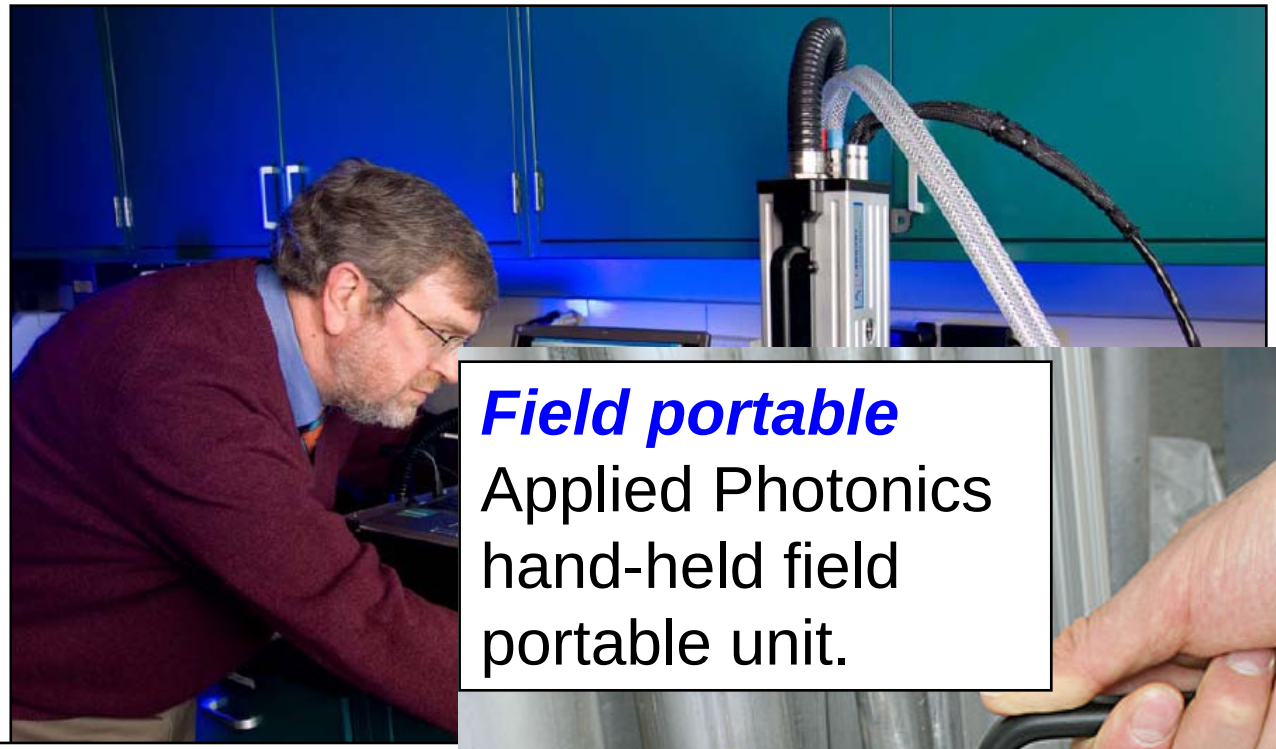
8 publications in Applied Physics Letters, Journal of Applied Physics, Applied Optics, Applied Spectroscopy, Spectrochimica Acta B, and others – confirmed by multiple other groups

Much remains to be done…

1. Making LIBS a realistic medical diagnostic (hardware/software)

2. Isolating bacteria from clinical specimens (blood? urine? CSF? saliva?) and concentrating them into the LIBS plasma

3. Benchmarking against gold-standards and other technologies on clinical isolates



Into the Lab!

We are communic
the Army Researc
equipment for tes
corporate quality
Andrzej Miziolek
Applied Photonics

Field portable

Applied Photonics hand-held field portable unit.

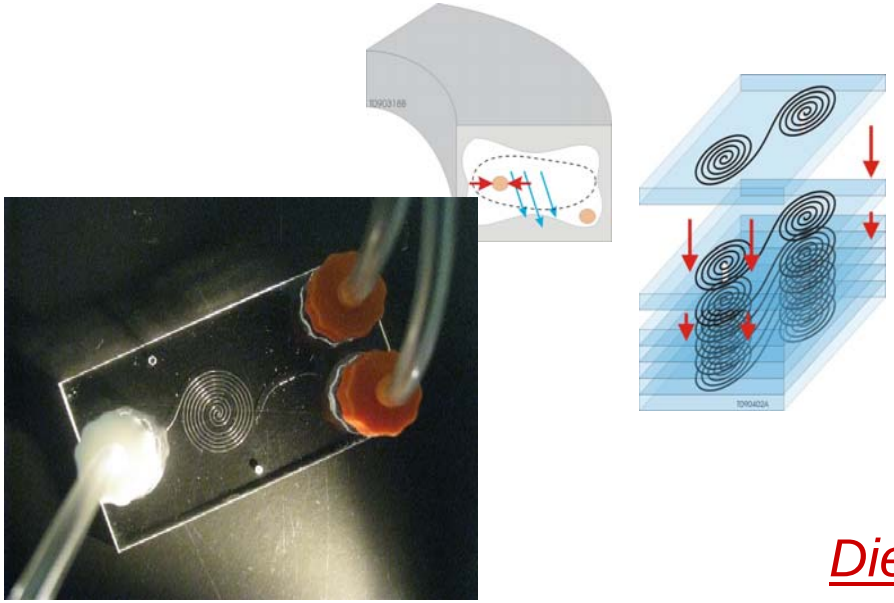
Much remains to be done…

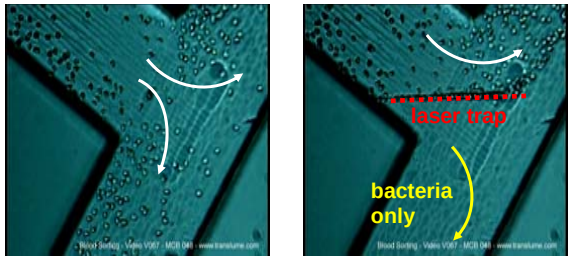
1. Making LIBS a realistic medical diagnostic (hardware/software)

2. Isolating bacteria from clinical specimens (blood? urine? CSF? saliva?) and concentrating them into the LIBS plasma

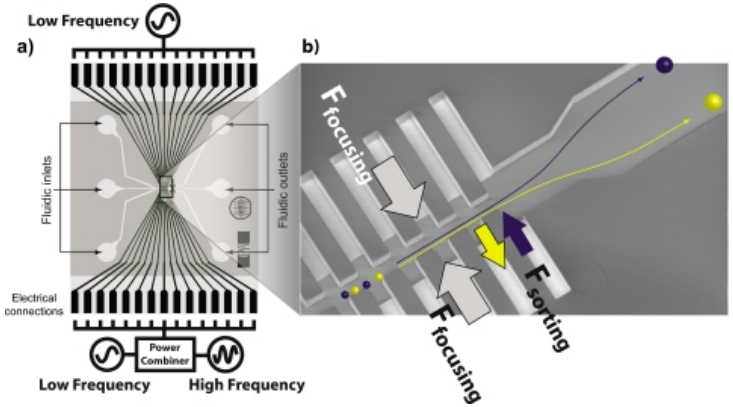
3. Benchmarking against gold-standards and other technologies on clinical isolates

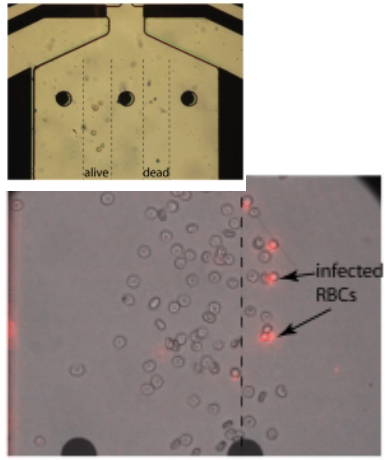
Microfluidic separation/concentration (Translume, Inc. Ann Arbor, MI)





Dielectrophoresis





PMC2917879A miniaturized continuous dielectrophoretic cell sorter and its applications
Ana Valero, Thomas Braschler, Nicolas Demierre, and Philippe Renaud
Biomicrofluidics. 2010 June; 4(2): 022807.

Much remains to be done…

1. Making LIBS a realistic medical diagnostic (hardware/software)

2. Isolating bacteria from clinical specimens (blood? urine? CSF? saliva?) and concentrating them into the LIBS plasma

3. Benchmarking against gold-standards and other technologies on clinical isolates

Bruker Daltonics

BRUKER

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)

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.

University of Windsor
thinking forward

INNOVATION.CA
CANADA FOUNDATION FOR INNOVATION | FONDATION CANADIENNE POUR L'INNOVATION

Work continues, with generous help from the University of Windsor, a Discovery Grant from NSERC, and a CFI-LOF grant (no success with CIHR yet)

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

Thank you for your attention!