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

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

Khadijia Sheikh, Russell Putnam, Andrew Daabous, Ryan Woodman, Daniel Trojan, Eric Lessard, Derek Gillies
University of Windsor, Department of Physics
bacteria are ubiquitous

10x more prokaryotic cells in your body than eukaryotic cells

E. coli

Staph. epidermidis

Staph. aureus

V. cholerae
Antibiotic-resistant infections among children on the rise

E. coli kills Idaho toddler; spinach probe

December 8, 2003

Staph Infection Kills Football Player
By Norm Jones, Newswatch 16, Scranton, PA

Eanout Product Recall Grows in Salmonella Scare

CDC: 756 ill from salmonella-tainted tomatoes
Suspicious powder at National Bank not dangerous, police say

A hazardous material crew from Windsor fire and ambulance personnel decontaminates inside a pencil case that had been delivered to the National Bank in Windsor after 4 p.m. two firefighters and another worker sifted through the found obFG

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)
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.”

“Too small?”
What’s the problem?

Are bacteria just too small to work with?

"Quantum Corral"

Scanning Tunneling Microscope image of individual iron atoms arranged intentionally on a copper surface in a circular ring, exposing quantum electron waves
Bacteria are 10,000x bigger!
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...

**New Lasers Fight Crime, Martians**

By Alexa Madrigal | February 16, 2010 | 6:20 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.
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.

…and Microbes???
1) laser interaction with the target

- 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 femtosecond 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)

- 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.
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…

E. coli from liquid specimen. Centrifuged than supernatant removed

about 500-1500 bacteria per sampling location

10 microliter
**bacterial composition**

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

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

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

<table>
<thead>
<tr>
<th>Element</th>
<th>% of fixed salt fraction</th>
</tr>
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<tbody>
<tr>
<td>Sodium</td>
<td>2.6</td>
</tr>
<tr>
<td>Potassium</td>
<td>12.9</td>
</tr>
<tr>
<td>Calcium</td>
<td>9.1</td>
</tr>
<tr>
<td>Magnesium</td>
<td>5.9</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>45.8</td>
</tr>
<tr>
<td>Sulfur</td>
<td>1.8</td>
</tr>
<tr>
<td>Iron</td>
<td>3.4</td>
</tr>
</tbody>
</table>
Cellulose Filter

filter

filter with bacteria

C

Na

Ar

Mg

Ca

Na

P

Ar
IR Nd:YAG laser
Echelle spectrometer
Sample chamber (w/ purge gas)
Microscope assembly
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…

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

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 communicating with other entities, (private companies, the Army Research Laboratory) to develop standardized equipment for testing in laboratories, emergency rooms, corporate quality control labs, diagnostic labs, etc. Here Dr. Andrzej Miziolek of ARL is shown testing a sample with their Applied Photonics prototype apparatus.

Field portable
Applied Photonics hand-held field portable unit.
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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
Much remains to be done…

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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.

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)
Thank you for your attention!