

Lasers to Rapidly Identify/Detect Pathogenic Bacteria

“Putting the B in CBRNE”

Steven J. Rehse *WSU, Dept. of Physics and Astronomy*

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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
CBC News



December 8, 2003

Staph Infection Kills Football Player

By Norm Jones, Newswatch 16, Scranton, PA

CNNhealth.com

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

Canada links Toronto plant to deadly listeriosis outbreak

The New York Times

Peanut Product Recall Grows in Salmonella Scare

By GARDINER HARRIS

Published: January 28, 2009

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

Antibiotic-resistant infections among children on the rise



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Superbug spreading



The severe drug-resistant strains of *Klebsiella pneumoniae* have been reported to the CDC by hospitals in at least 35 states and Washington, D.C.

By Anne Godlasky, USA TODAY; Source: Centers for Disease Control and Prevention

Drug-resistant 'superbugs' hit 35 states, spread worldwide

Updated 9/17/2010 5:05 PM | Comments 466 | Recommend 46 | E-mail | Save | Print | Reprints & Permiss

MYSTERIOUS POWDER INVESTIGATED

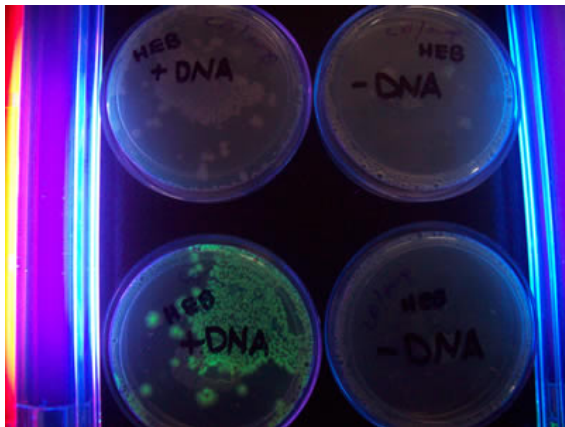


NICK BRANCACCIO/The Windsor Star

A hazardous materials team with Windsor Fire Services take readings and samples from the contents of a recycling box at the Transit Windsor garage located beside the Essex-Windsor Solid Waste Authority Central Avenue transfer station on Tuesday. Windsor police, fire and ambulance personnel descended on the garage on North Service Road Tuesday after an employee discovered white powder inside a pencil case that had been left behind on a bus. Police said an employee found the pencil case while cleaning the bus. Just after 4 p.m., two firefighters donned white hazmat suits, rubber boots, oxygen tanks and masks to prepare to handle the material. The workers sifted through the found objects and took samples of the powder to be examined by police. Police have not yet determined the origin or makeup of the powder. It has been taken to a laboratory in Etobicoke for testing.

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



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

What can we do?

Although there are several alternative approaches to solving this problem, we feel the use of laser light is one of the best.

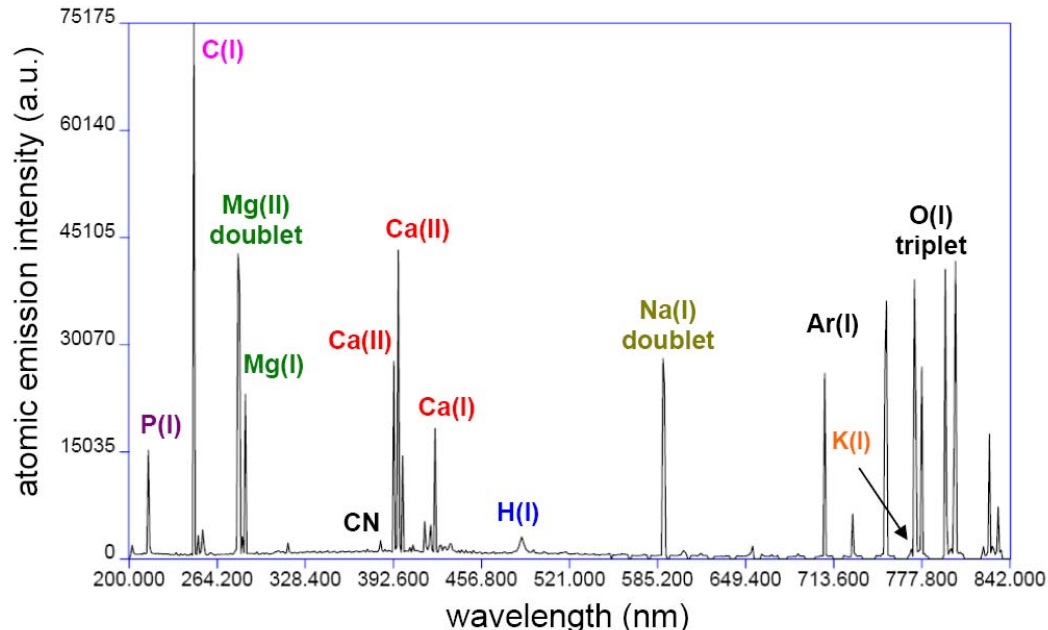
We want to go from this...



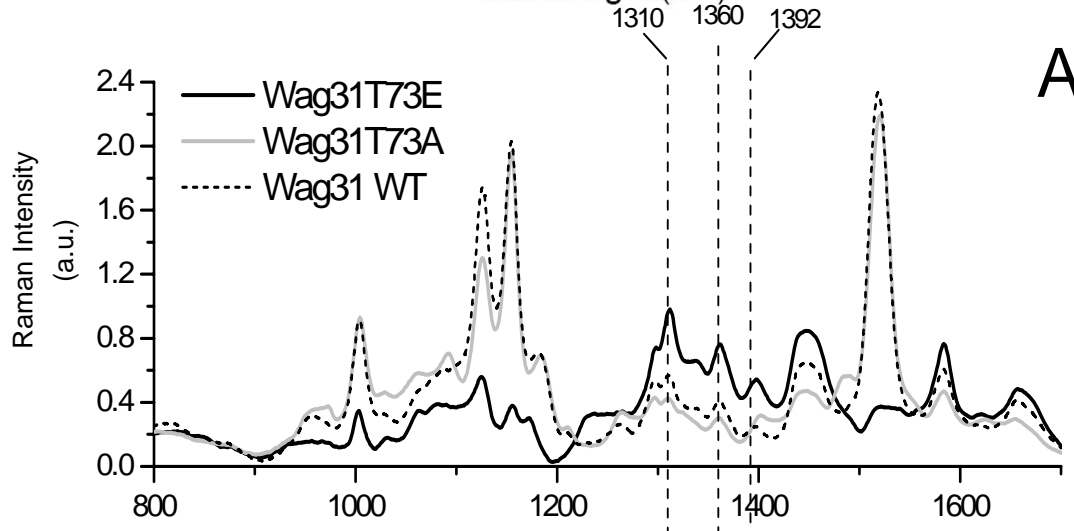
... to this!



Bacteria don't have barcodes, but using lasers we can obtain unique patterns or fingerprints...



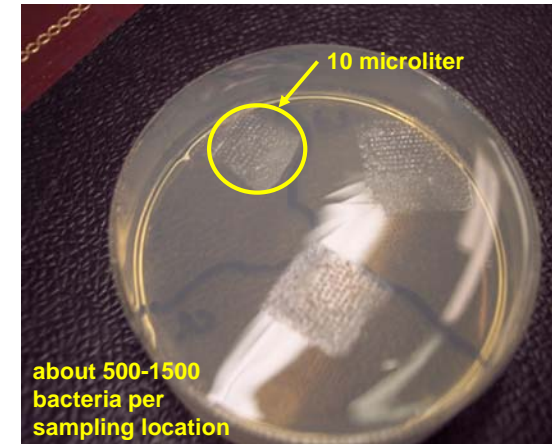
Laser-induced breakdown spectroscopy can reveal a bacterium's **atomic composition** which provides a unique spectral fingerprint in real-time



Raman spectroscopy can reveal a bacterium's **molecular composition** which provides a unique spectral fingerprint in real-time

Advantage of using laser-based methods

- identifications made quickly
 - (under 5 minutes, under 1 second?)
- low cell count necessary
- insensitive to contamination
- safety
 - (dead bacteria, stand-off distances)
- non-experts can use them easily
- no biochemicals/consumables
- computerized diagnoses



Who needs these techniques? (huge market demand)

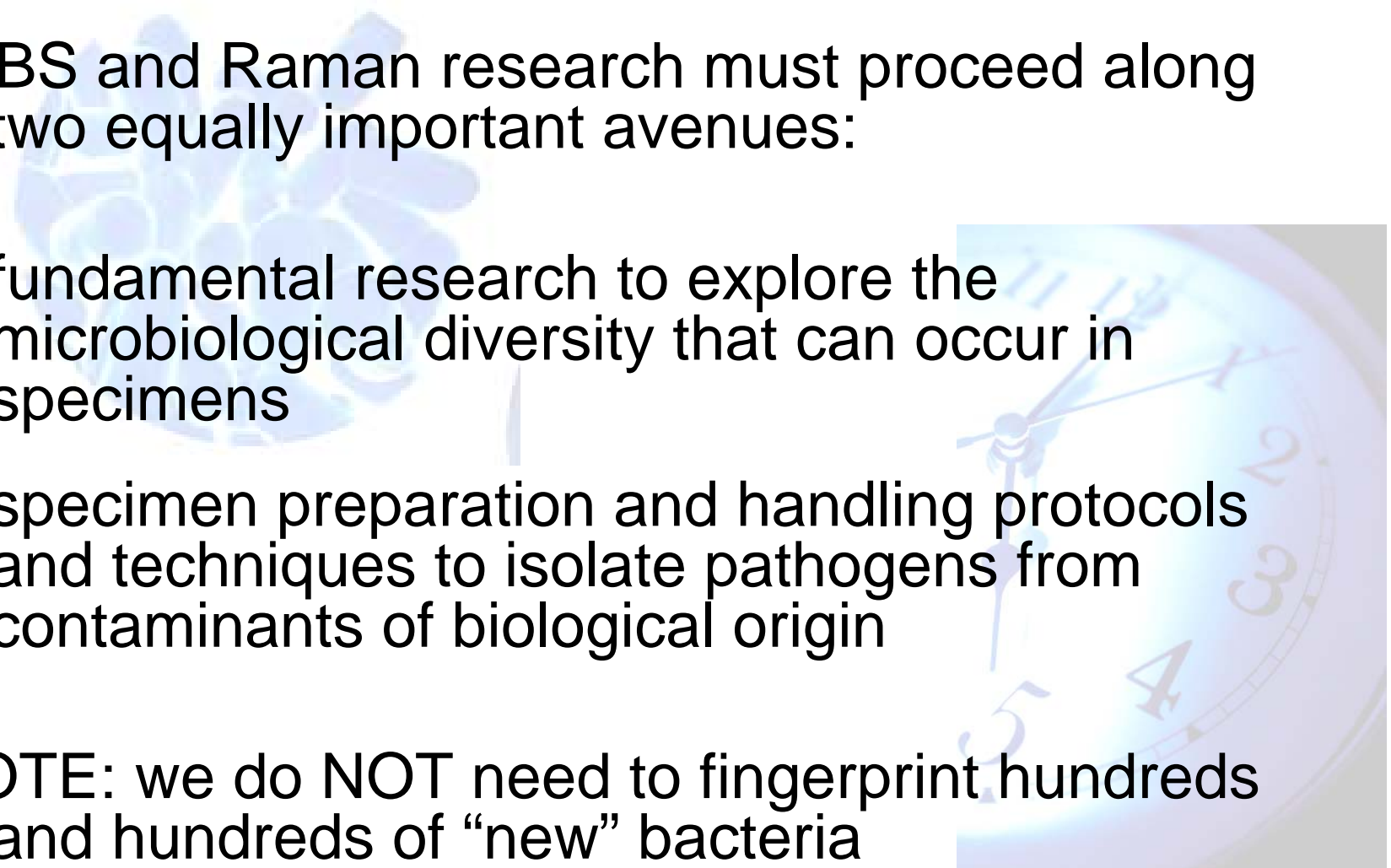
- food / beverage corporations
- hygiene compliance officers / FDA
- clean water utilities / EPA
- first responders
- **clinicians: hospitals / physicians / CDC**
- military medicine
- NASA / exo-biologists

We Must Proceed, and Faster...

LIBS and Raman research must proceed along two equally important avenues:

- fundamental research to explore the microbiological diversity that can occur in specimens
- specimen preparation and handling protocols and techniques to isolate pathogens from contaminants of biological origin

NOTE: we do NOT need to fingerprint hundreds and hundreds of “new” bacteria



Thank you

- My collaborators

***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*

***Tom Haddock** Translume, Inc., Ann Arbor, MI*

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

- My students

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

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

***Caleb Ryder** WSU, Dept. of Physics and Astronomy*

Back-up slides

LIBS: The Wayne State Team has already demonstrated...

LIBS spectral fingerprint is:

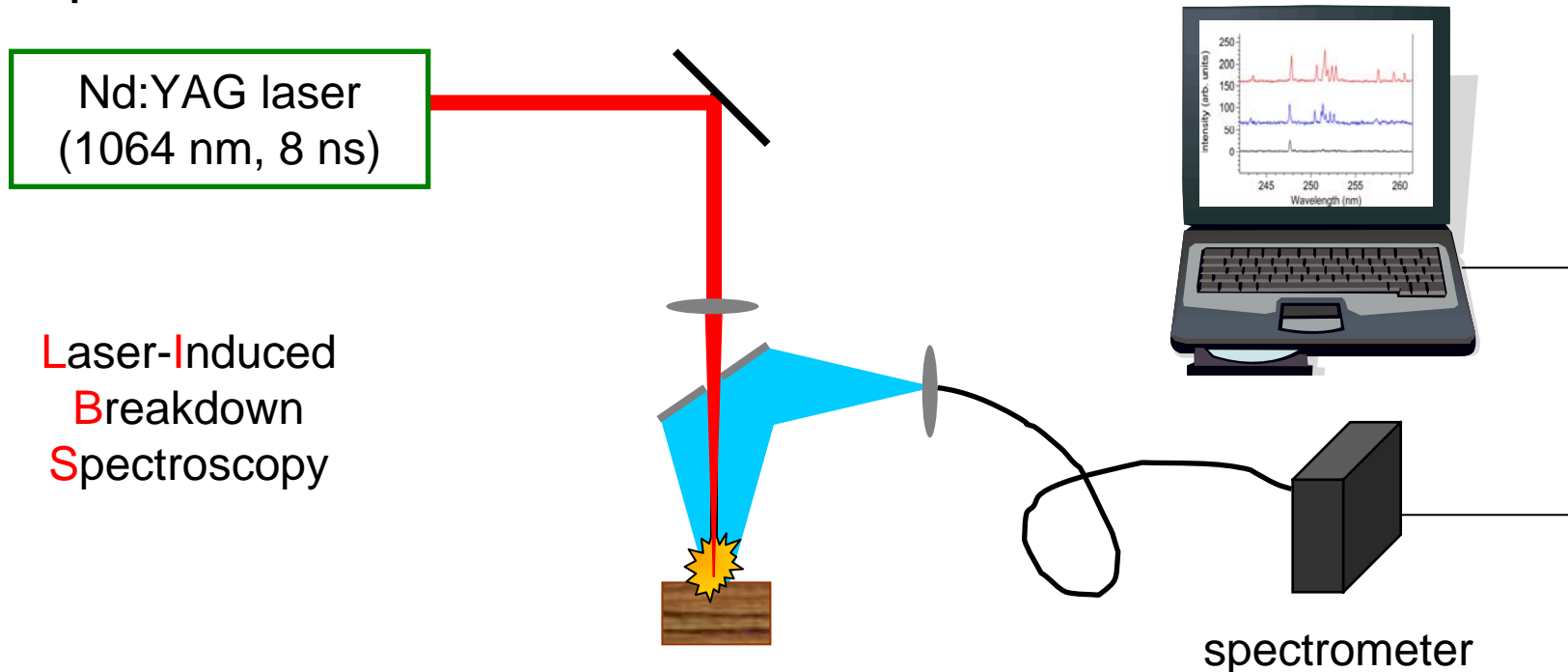
- growth-medium independent
- independent of state of growth (how “old” the bacteria are)
- independent of whether the bacteria are live or dead (or inactivated by UV light)
- obtainable even when other types of bacteria or contaminants are present (mixed samples)
- obtainable even when other biochemicals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

The Raman spectral fingerprint:

- is more sensitive than a LIBS fingerprint
- requires more bacteria (lower SNR)
- can identify biochemical changes in closely-related mutants due to protein phosphorylation
- can be obtained from cell envelope fractions
- can easily discriminate multiple *E. coli* strains
- can be obtained from proteins
- can be used to track metabolic or carbolic activity

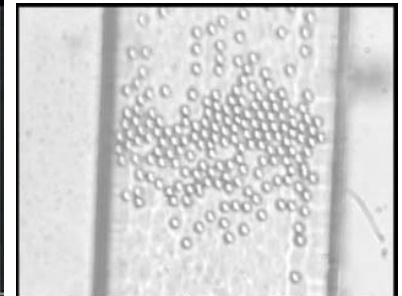
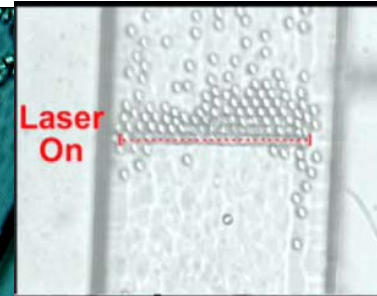
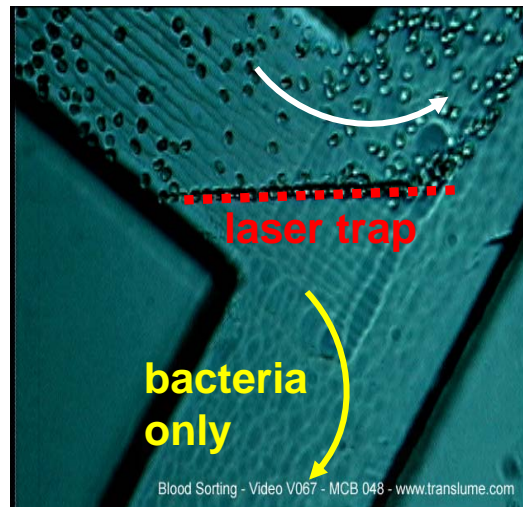
EMMA: Elemental Multivariate Microbiological Analysis

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

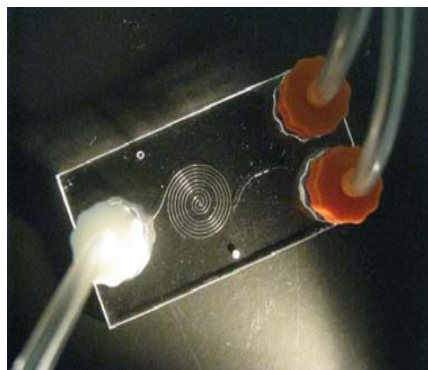


LIBS Spectrum is like a Bar Code- Unique for Each Sample

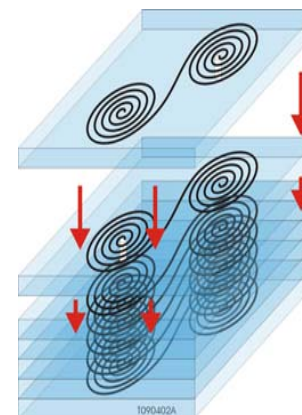
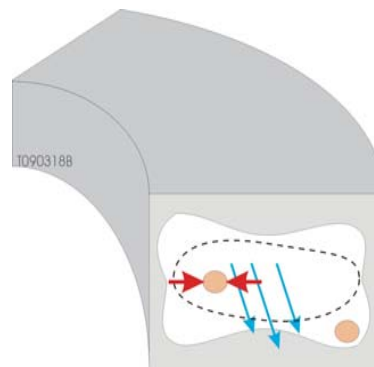
Microfluidic separation/concentration (Translume, Inc. Ann Arbor, MI)



optical trap-based separation of heavier cells from lighter cells



monolithically fabricated devices in glass



hydrodynamic (microfluidic) separation of heavier cells from lighter cells