Using Lasers to Detect and Identify Bacteria: An Interdisciplinary Project

presented to the Science City Café, Wednesday, October 19th, 2011

Steven J. Rehse

The University of Windsor
Department of Physics

University of Windsor
thinking forward

Why is this "Interdisciplinary"?

Using Lasers

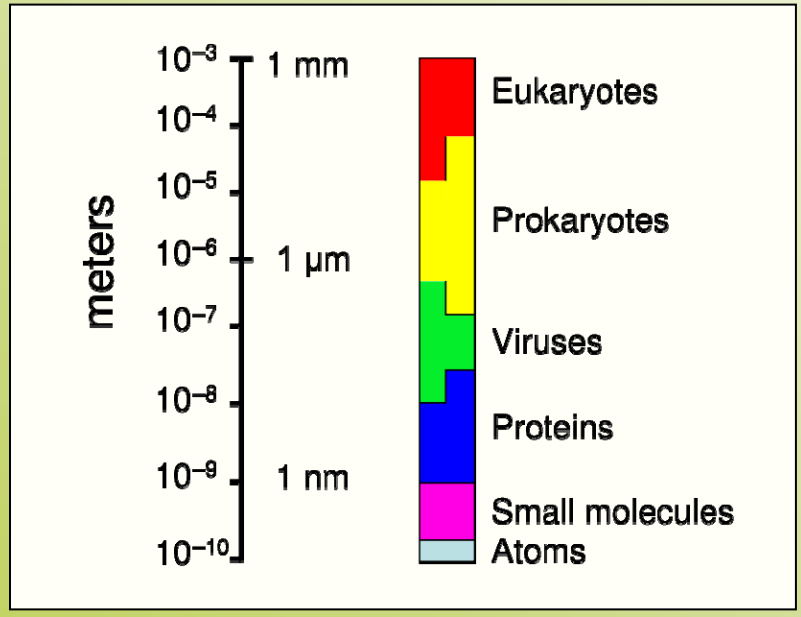
ify Bacteria

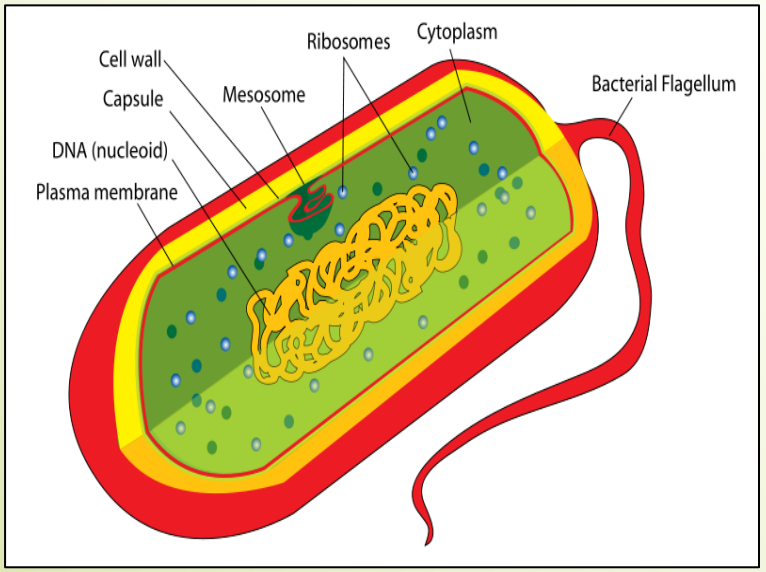
Interdisciplinary Science

obiology

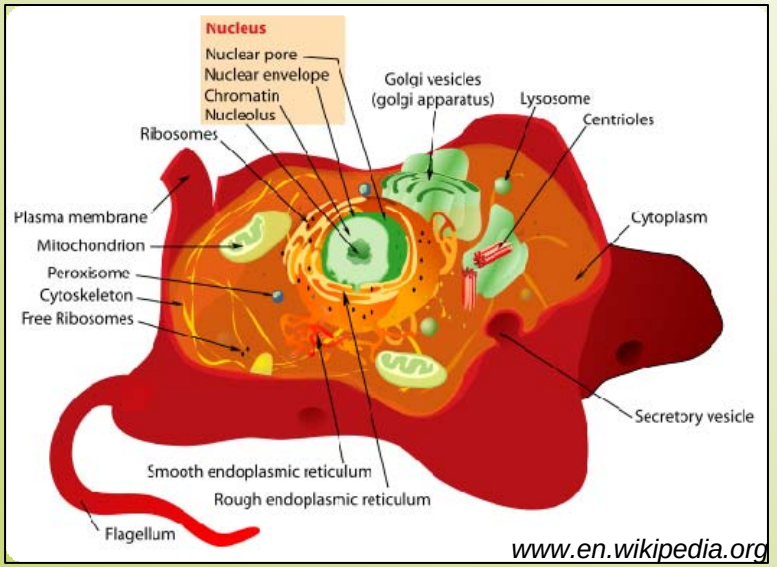
Types of Cells

Prokaryote

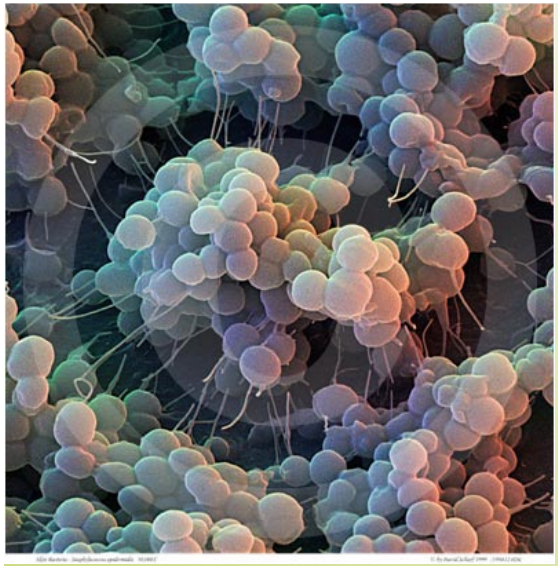




Eukaryote

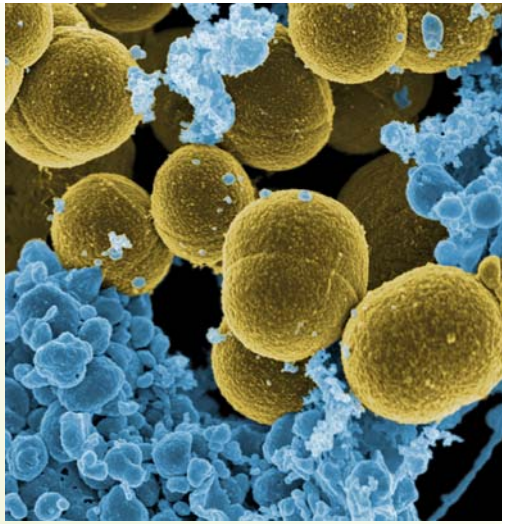


www.en.wikipedia.org



Staph. epidermidis

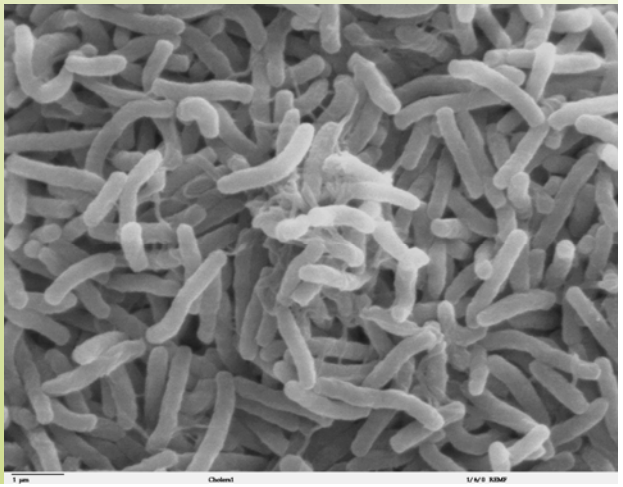
Staph. aureus



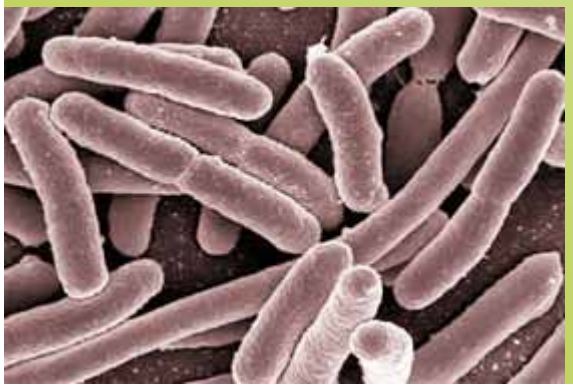
bacteria are ubiquitous

10x more prokaryotic cells in your body than eukaryotic cells

V. cholerae



E. coli



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

Antibiotic-resistant infections among children on the rise

CNNhealth.com

iversity of Windsor

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

Canada links Toronto plant to deadly listeriosis outbreak

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E. coli kills Idaho toddler; spinach pla probed

Updated 10/5/2006 8:57 PM ET

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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

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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

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CDC: 756 ill from salmonella-tainted tomatoes

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.

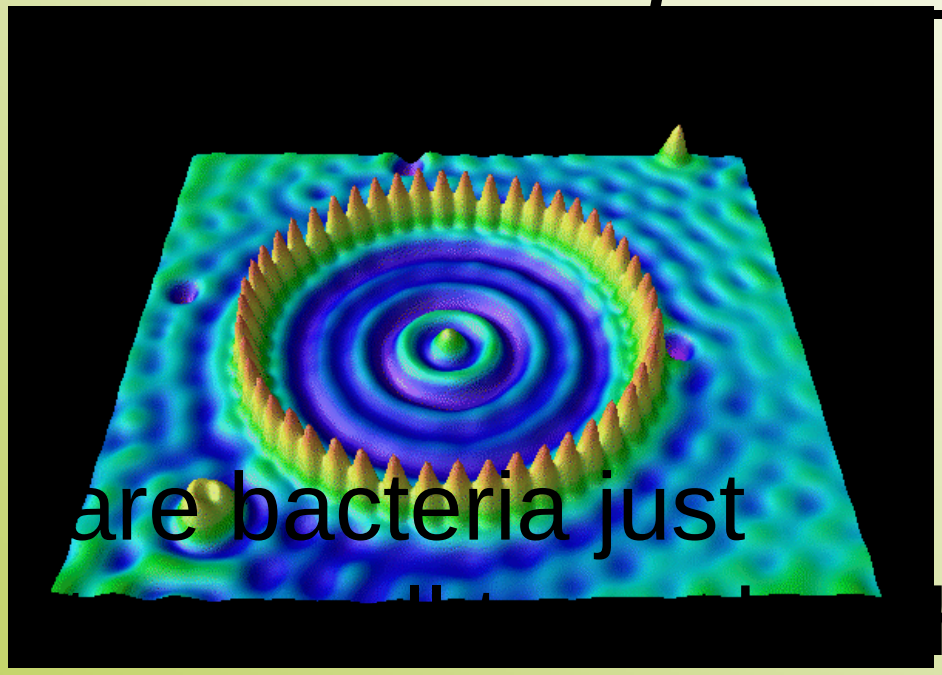
So why?

"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?

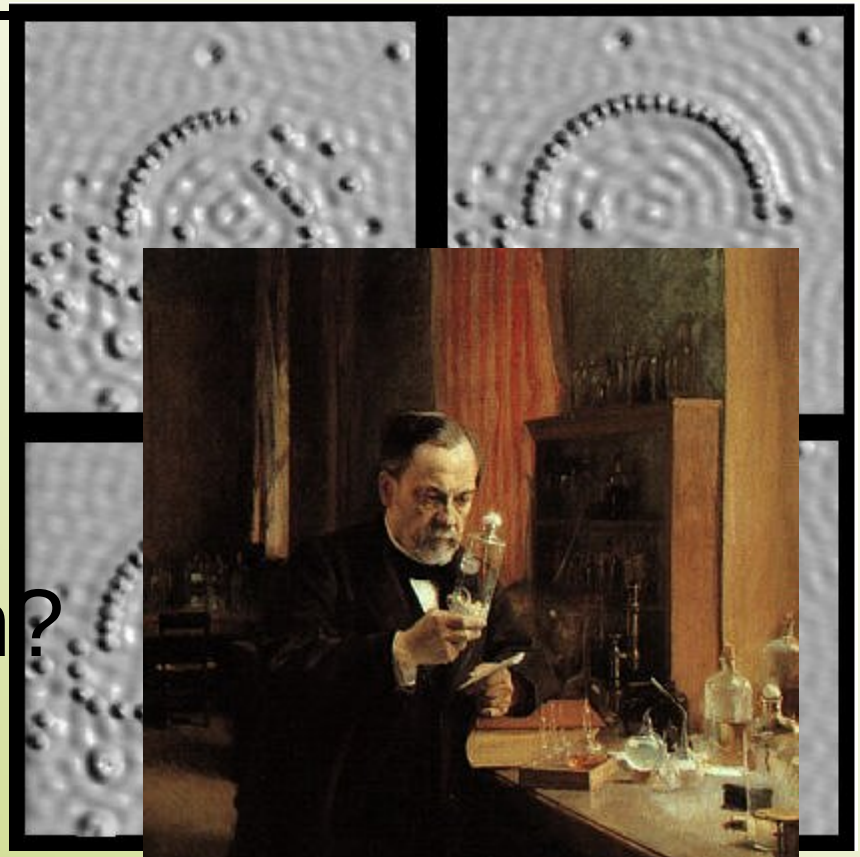


are bacteria just

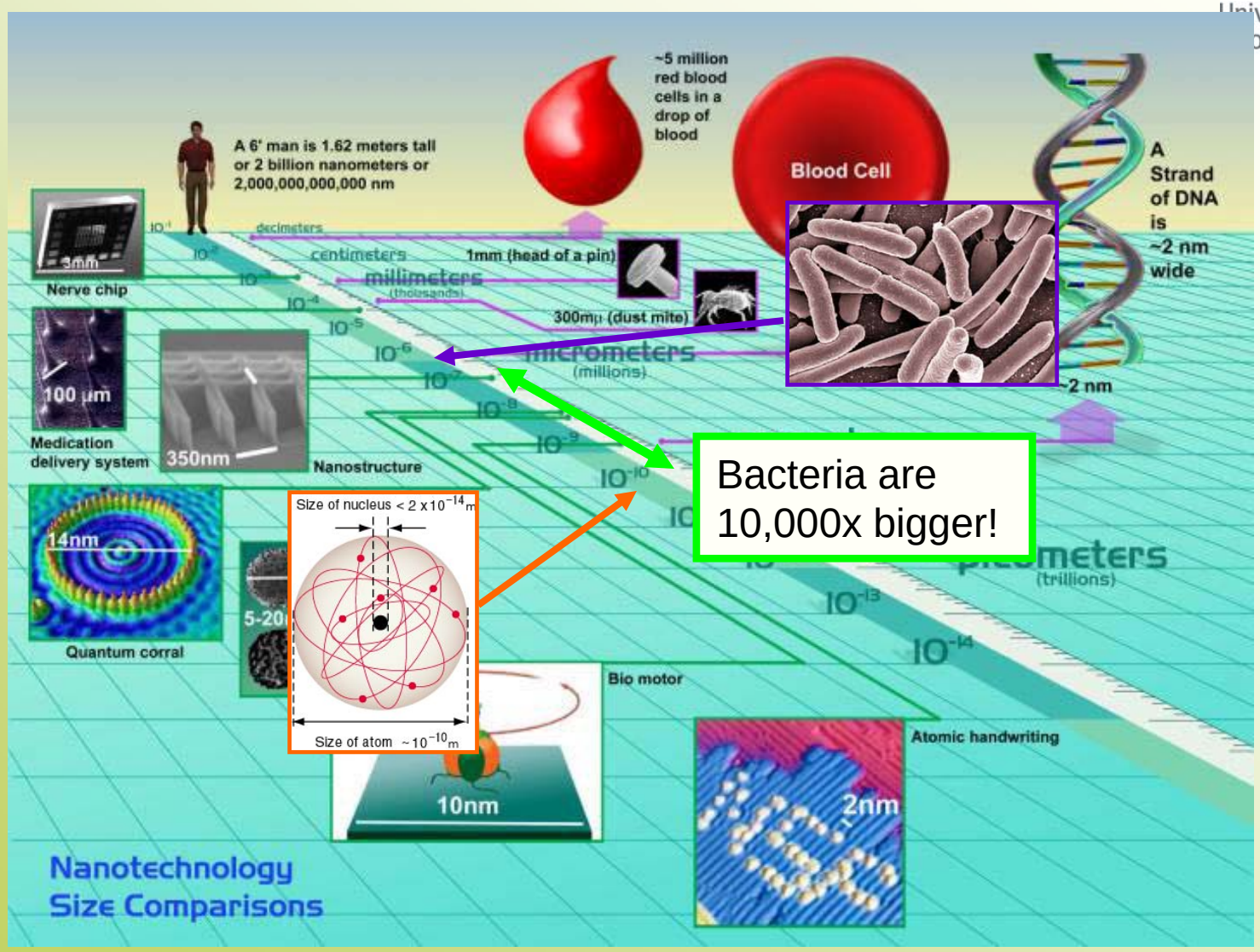
h?

"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 ing



From “Nanopedia” at Case Western University

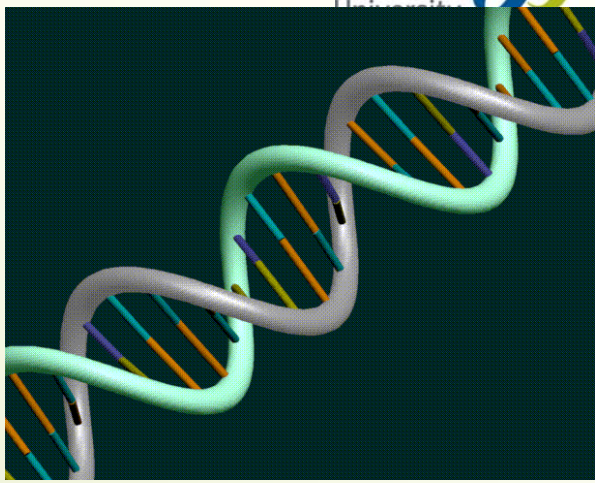
If it's not the size, it must be our methods

How do we identify bacteria?

4 ways

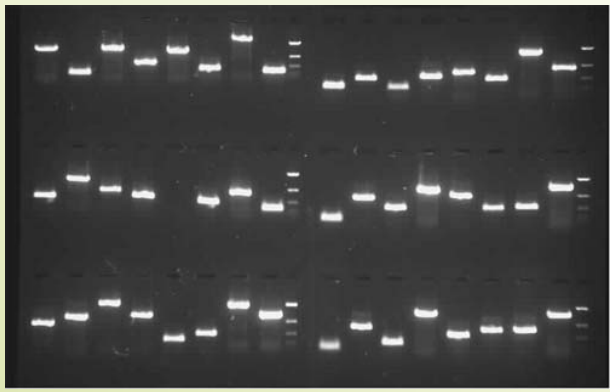
- genetic
- serological (antigenic)
- microbiological
- compositional

genetic



- all living organisms have a unique genetic code, contained in their DNA – a long organic macro-molecule
- the DNA is much smaller than the bacterium and it must be harvested by chemical methods

requires

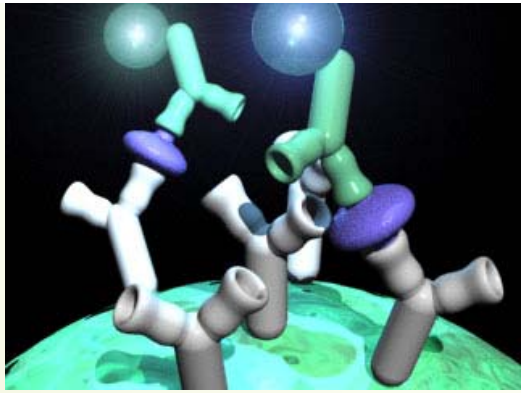


- prior knowledge of part of the genetic sequence
- one strand must be amplified to many

drawbacks

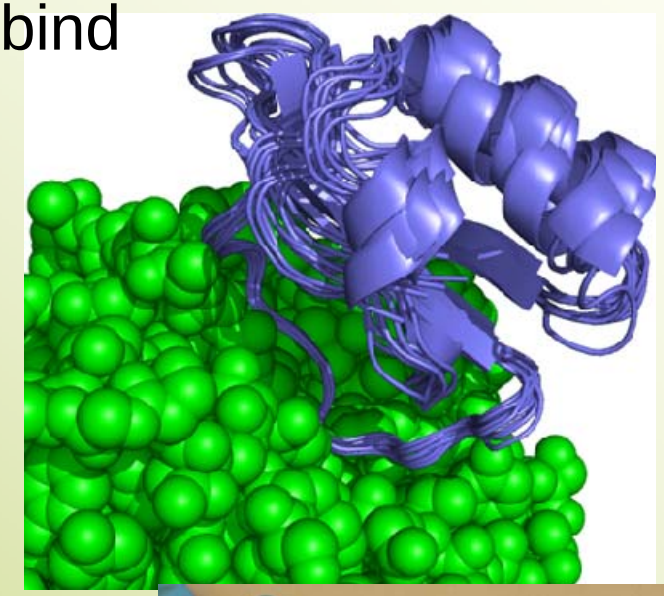
- amplification time (multiple generations needed)
- nonspecific reactivity
- still need to do gel electrophoresis
- very contamination sensitive

serological



- bacteria have unique combinations of molecules (antigens) on their outer surface

- antibodies can be used which uniquely bind to only those antigens

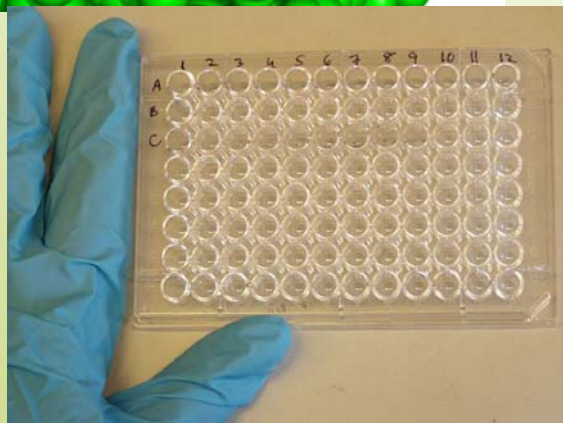


requires

- prior knowledge of serology (surface antigens)

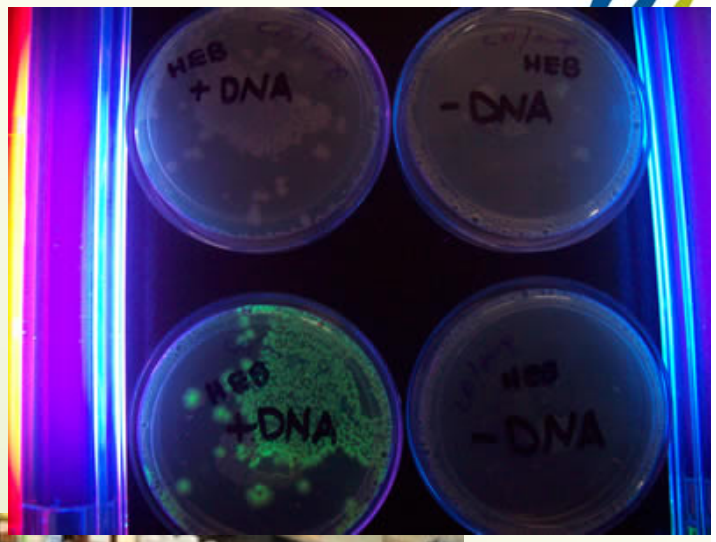
drawbacks

- 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
- prior clinical knowledge (case-history)

drawbacks

- slow/labor intensive
- requires experts

compositional

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- bacteria have unique combinations of atoms
- bacteria have unique combinations of molecules
- bacteria have unique combinations of proteins

requires

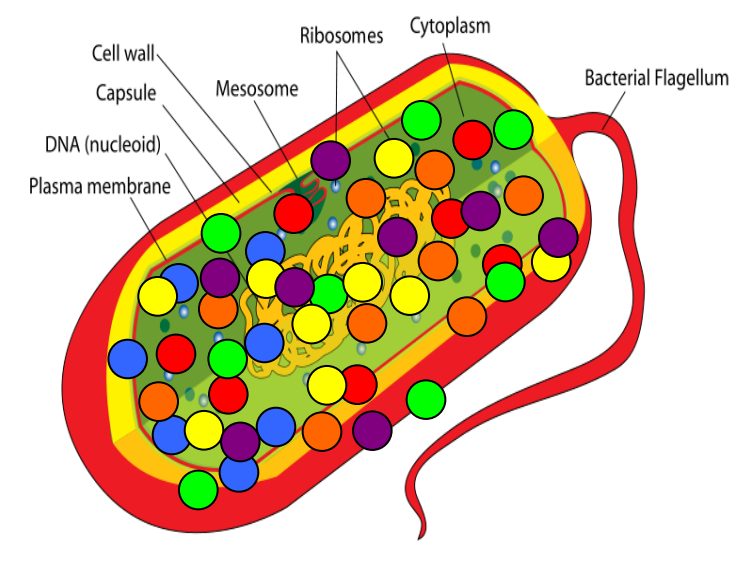
- no prior knowledge of serology (surface antigens)
- no prior knowledge of genetic sequence
- no consumables (hopefully)
- no expertise

drawbacks

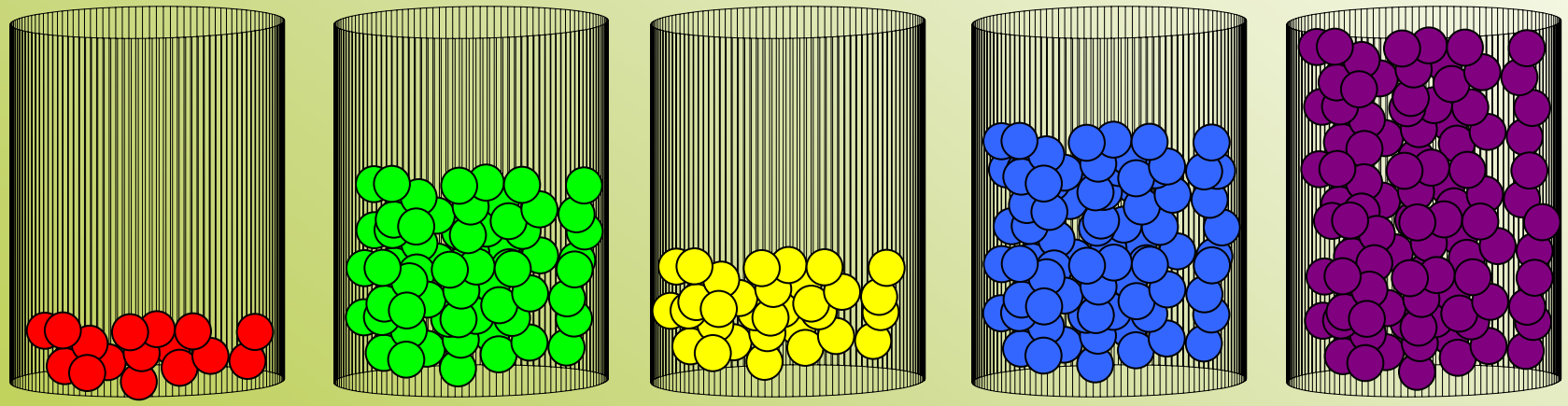
- sensitivity (no amplification)
- hardware probably expensive (relative)
- specificity?

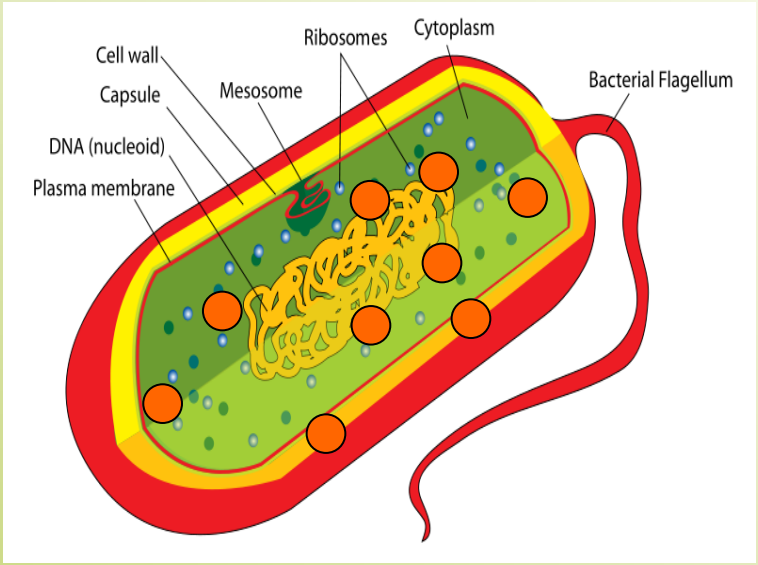
So my question is/was… (*hypothesis)*

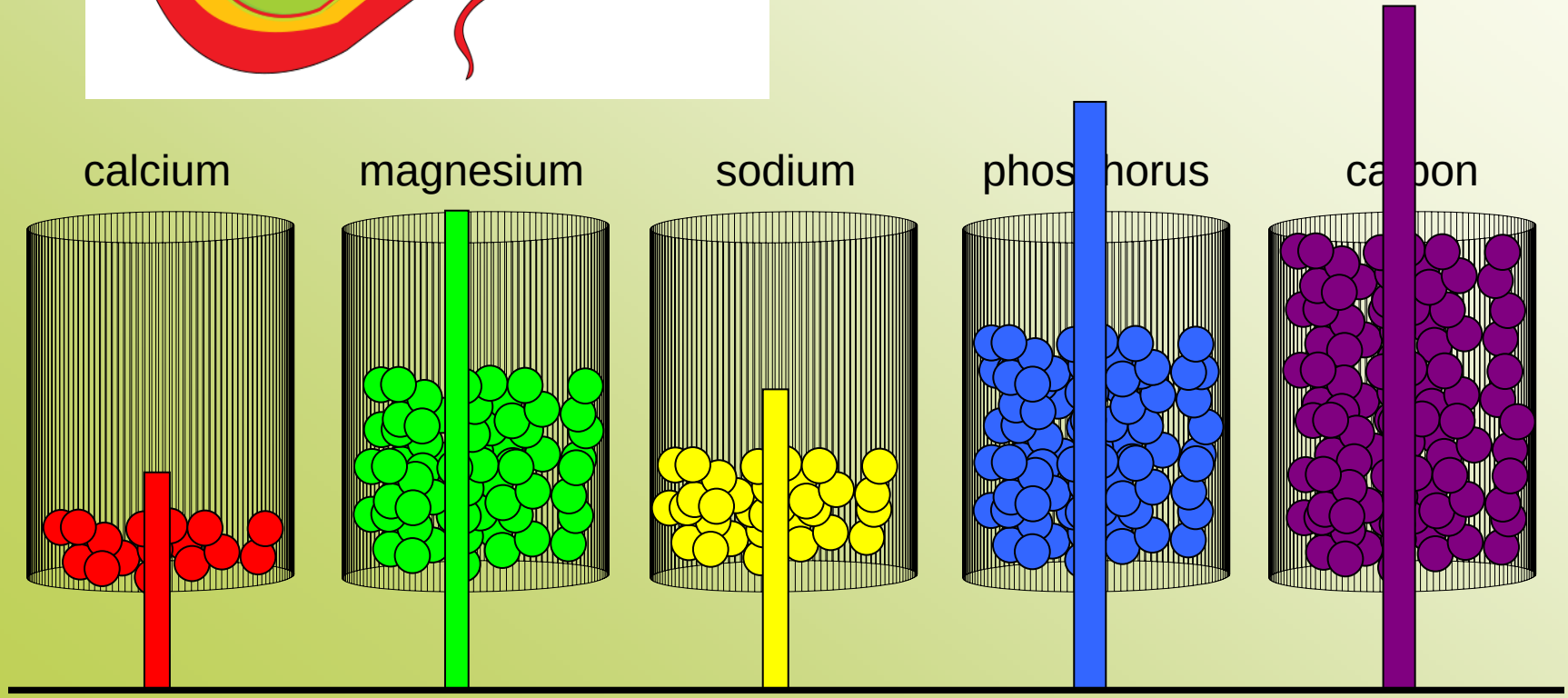
Can a bacteria be rapidly identified by knowing the amount and type of all the atoms in it?



calcium magnesium sodium phosphorus carbon







We want to go from this…

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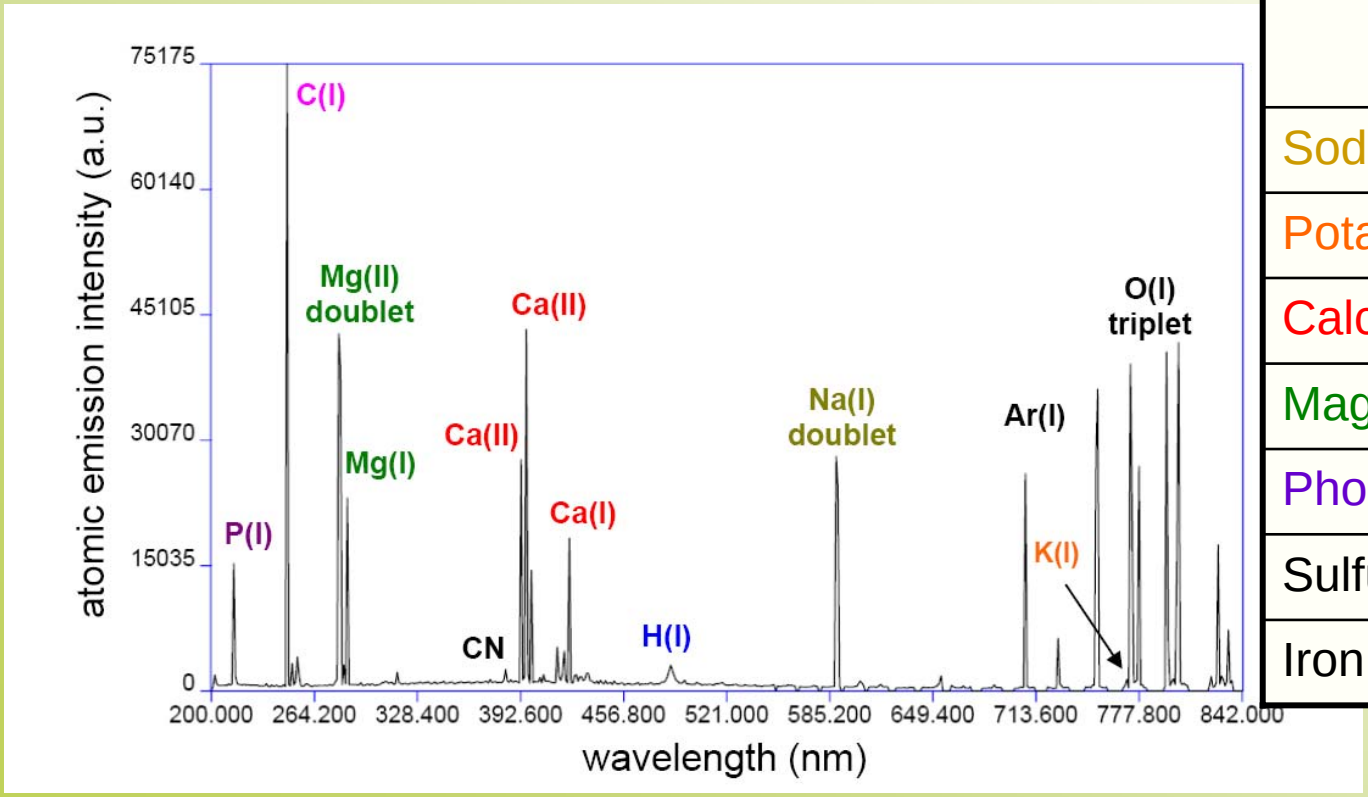
… to this!

What can we do?

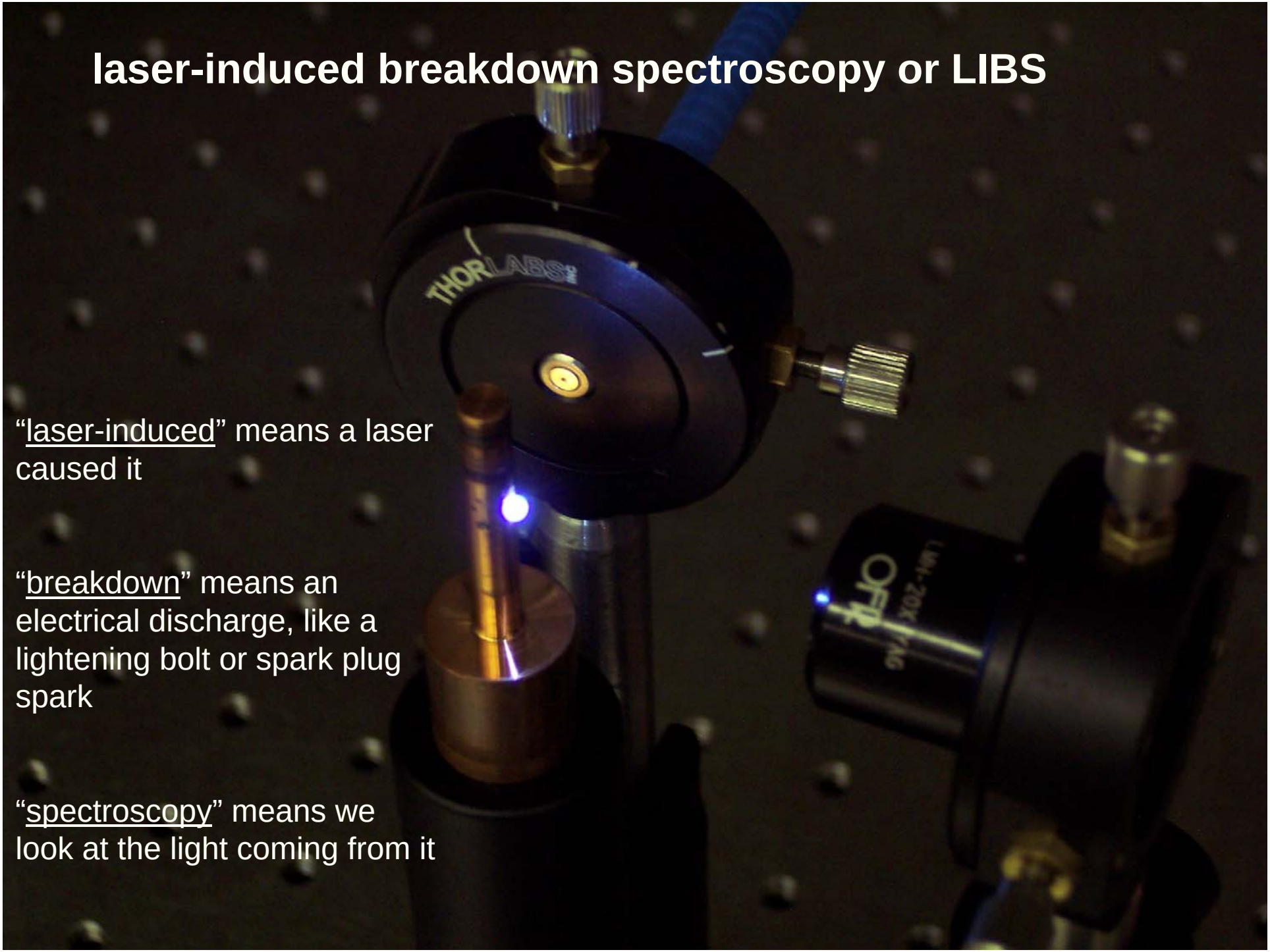
Although there are several alternative approaches to solving this problem, we feel the use of laser light is one of the best.

The laser-based method I am proposing produces just such a bar-code/graph!



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

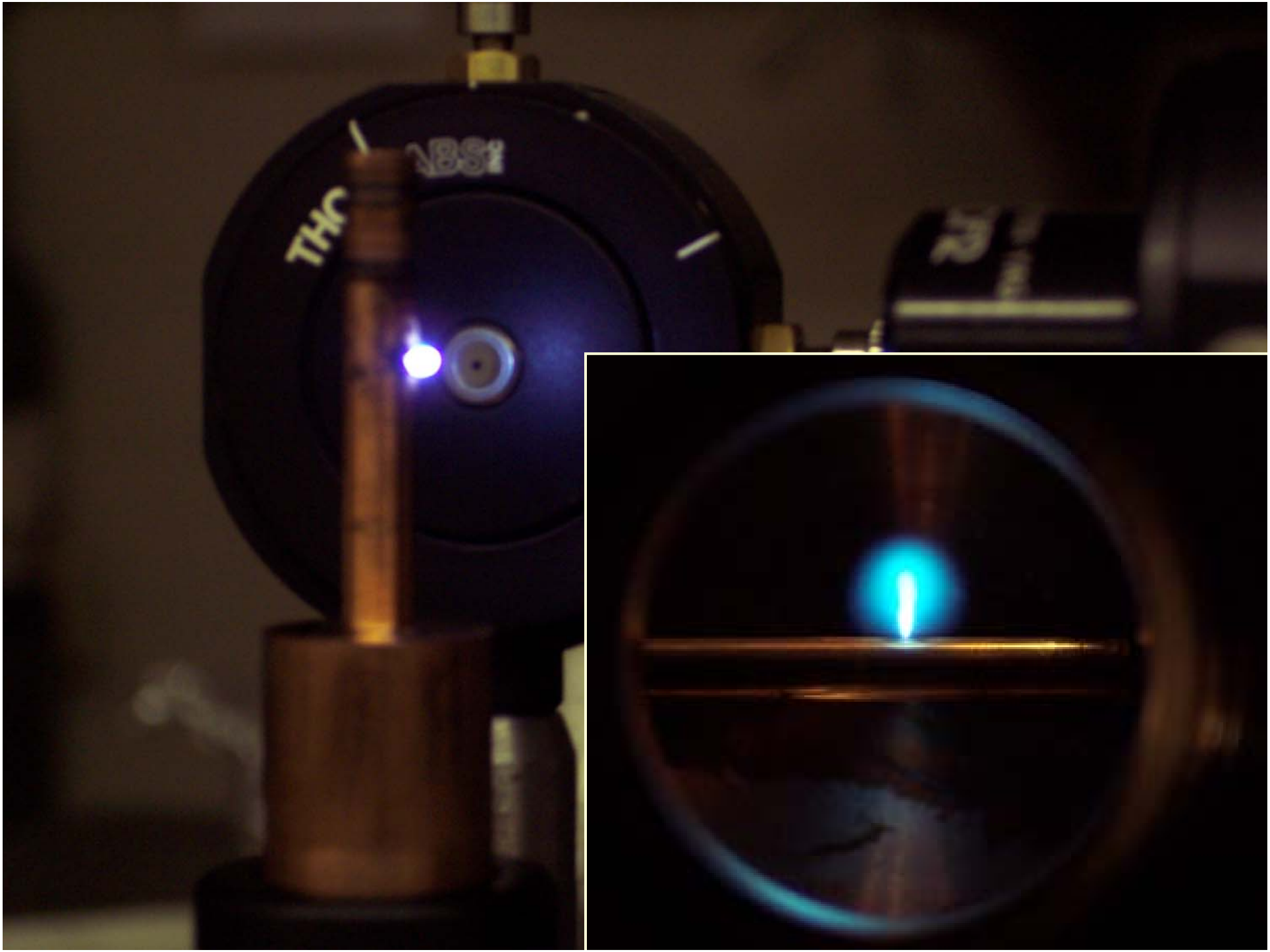
laser-induced breakdown spectroscopy or LIBS



"laser-induced" means a laser caused it

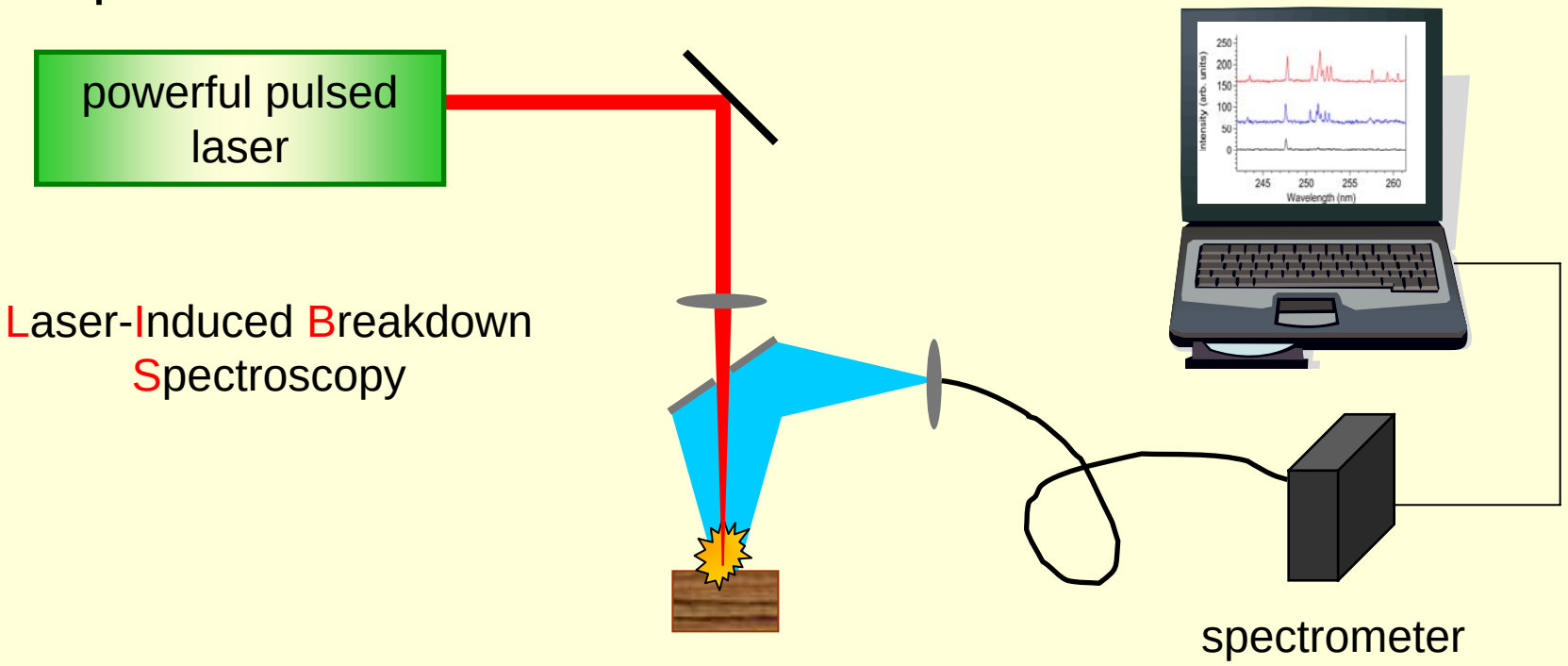
"breakdown" means an electrical discharge, like a lightening bolt or spark plug spark

"spectroscopy" means we look at the light coming from it

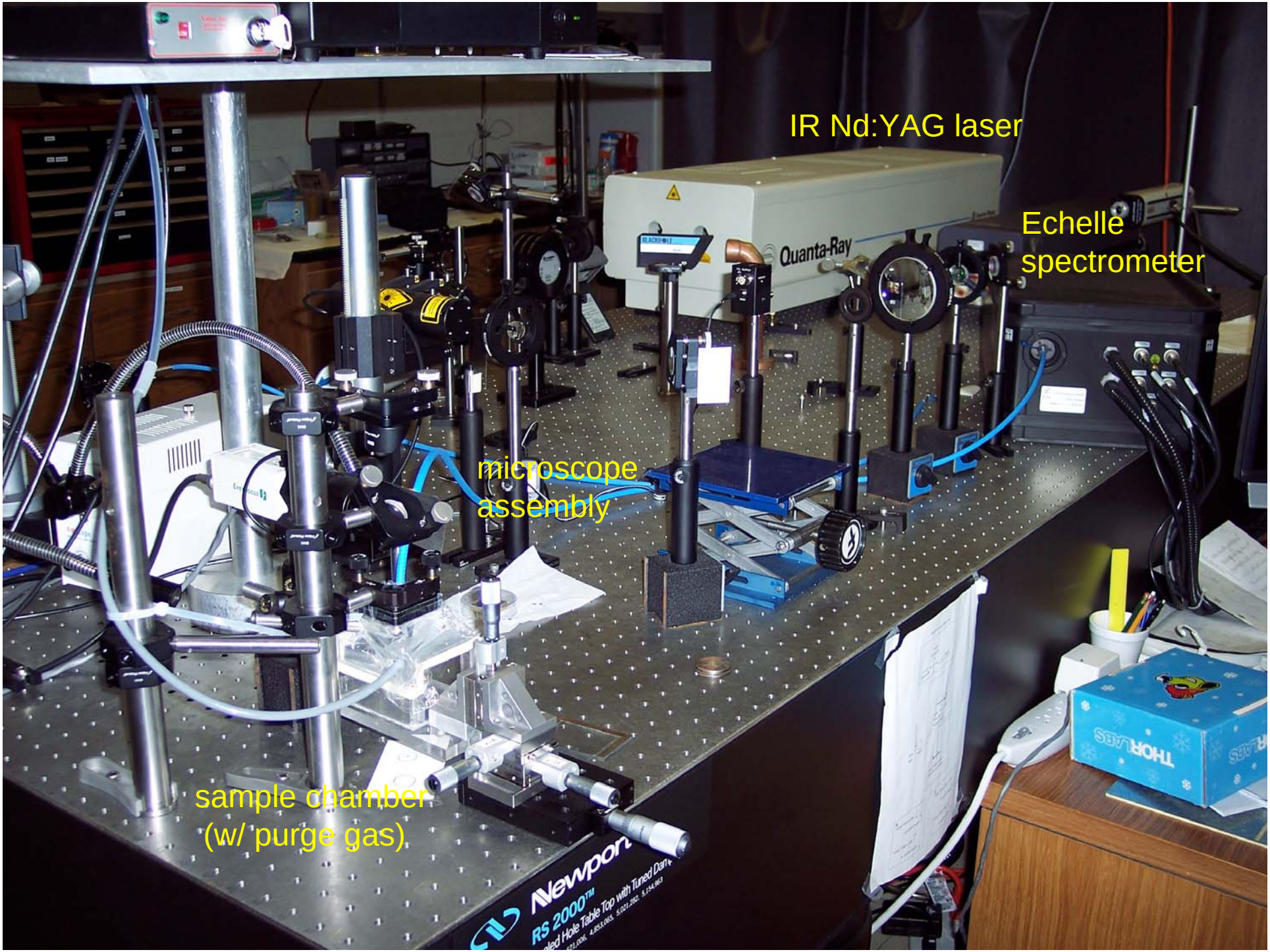


EMMA: Elemental Multivariate Microbiological Analysis

- 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


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

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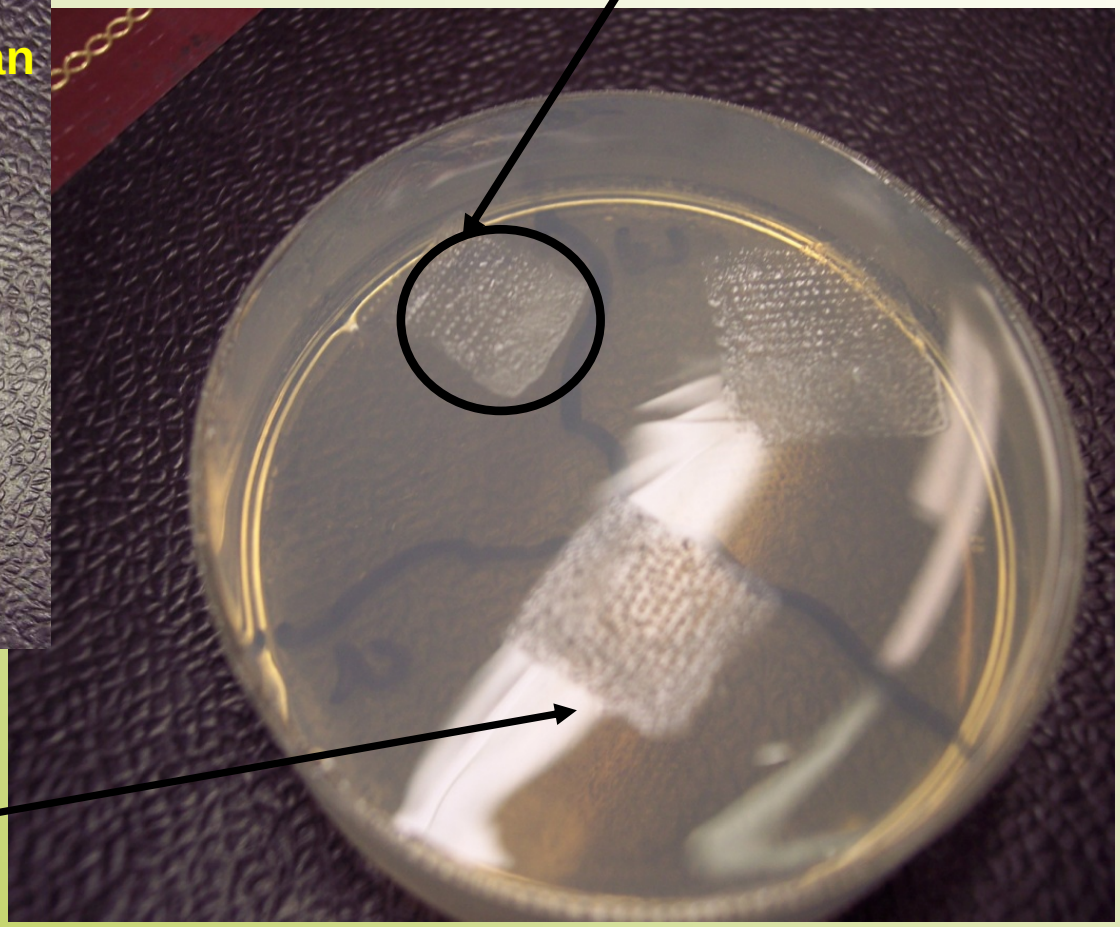
how we do it…

10 microliter



about 500-1500 bacteria per sampling location

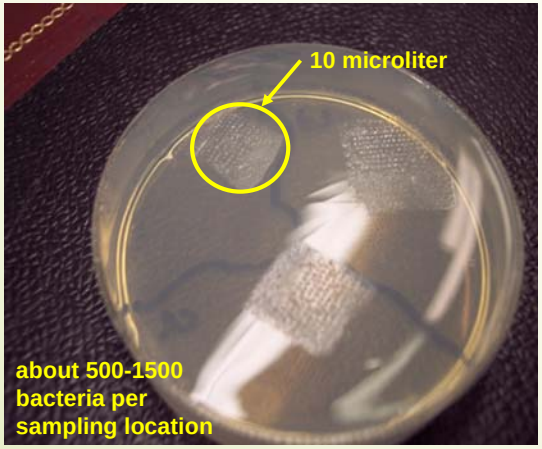
LIBS: The University of Windsor team has already demonstrated…

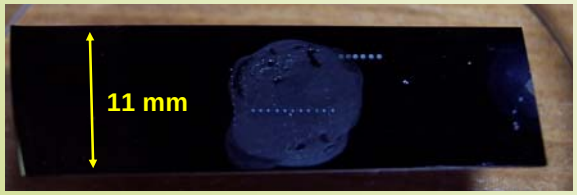
LIBS spectral fingerprint:

- does NOT depend on what the bacteria “eats”
- does NOT depend on how “old” the bacteria are
- is independent of whether the bacteria are alive or dead (or inactivated by UV light)
- is obtainable even when other types of bacteria or contaminants are present (mixed samples)
- can be obtained directly from bacteria in a urine sample
- is capable of strain discrimination
- can be obtained from about 500 bacteria (probably just one!)

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)

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- food / beverage corporations
- hygiene compliance officers / FDA / Canadian Food Inspection Agency
- clean water utilities / EPA
- first responders
- clinicians: hospitals / physicians / CDC / Public Health Agency of Canada
- military medicine
- NASA / exo-biologists

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

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

Head's-up display

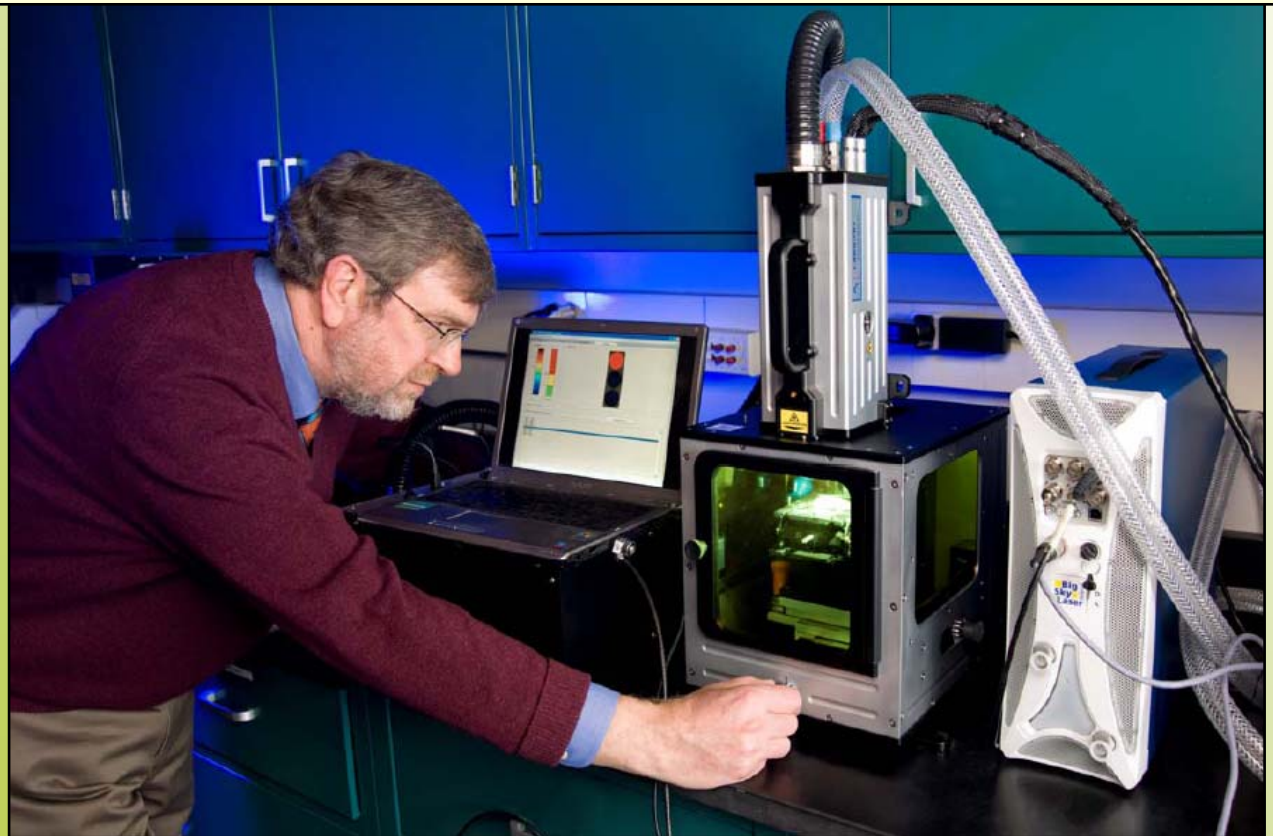
Hand-held probe contains laser, joystick for control, and focus optics

Microplasma/ LIBS Event

courtesy of Ocean Optics.

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.



courtesy of Applied Photonics Ltd, U.K.

ST-LIBS Gen 4 at Hidden Valley, NTC, Fort Irwin, California
(Published with permission of U.S. Army, National Training Center, Fort Irwin)
Image 13 of 23

CLOSE ✕

Laser plasma on clay target at 50 metres range (Hidden Valley, NTC, December 2007)
(Published with permission of U.S. Army, National Training Center, Fort Irwin)
Image 15 of 23

CLOSE ✕

courtesy of Applied Spectra, Inc.

the new “Mars Science Laboratory” (MSL), Mars Rover “Curiosity”, is going to Mars on Nov. 25th, 2011

http://mars.jpl.nasa.gov/msl/

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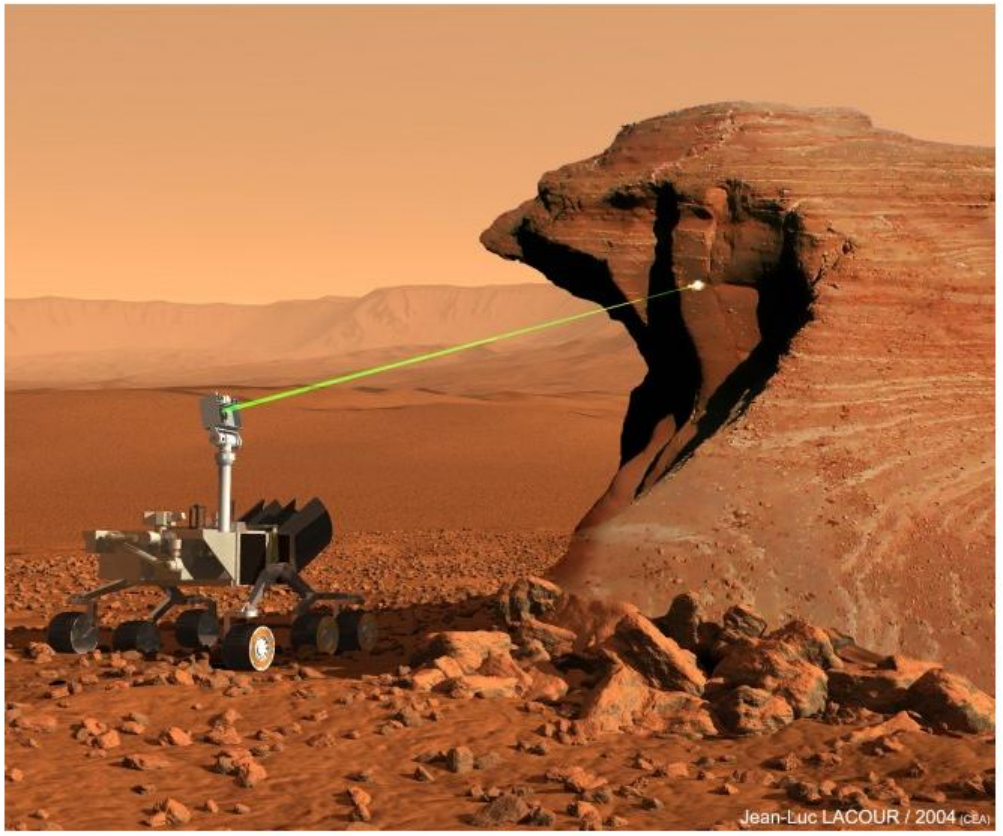
By Alexis Madrigal February 16, 2010 | 6:26 pm | Categories: Physics, Space



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.

Where I Think We Can Be in Under Five Years

(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*.

And on into the future…

http://www.uwindsor.ca/people/rehse/

http://www.arl.army.mil/www/default.cfm?page=247

Google search for "us army libs"

Thank you.

Questions?