Laser-Induced Breakdown Spectroscopy (LIBS) for the Rapid Identification and Classification of Pathogenic Bacteria

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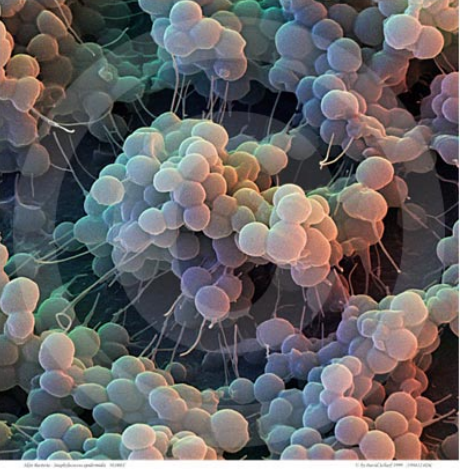
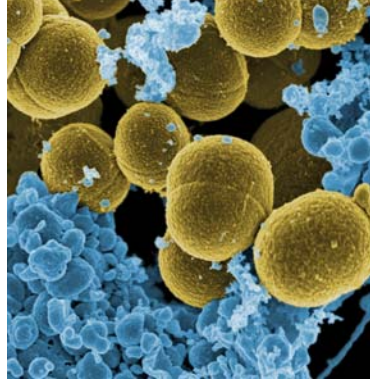


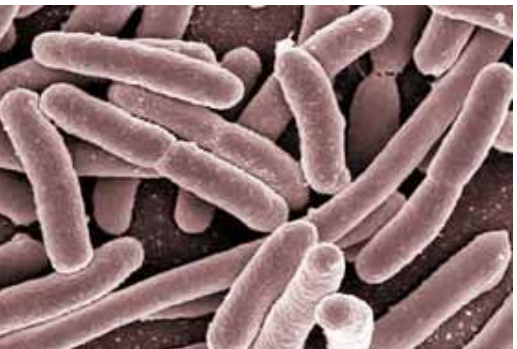
Staph. epidermidis

Staph. aureus

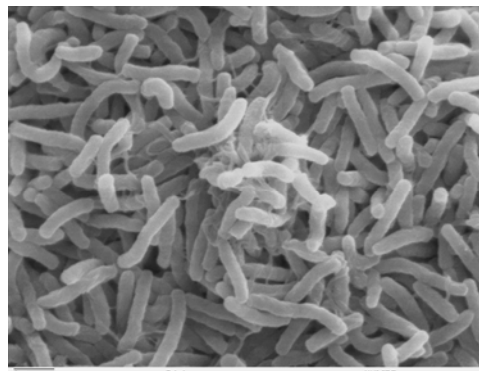


there is an urgent need right now in the military, civilian (hospital, food processing, environmental), and first responder communities for a "…rapid point-of-care (multiplex?) diagnostic for disease-causing pathogens."

E. coli



V. cholerae



How do we identify bacteria?

4 ways

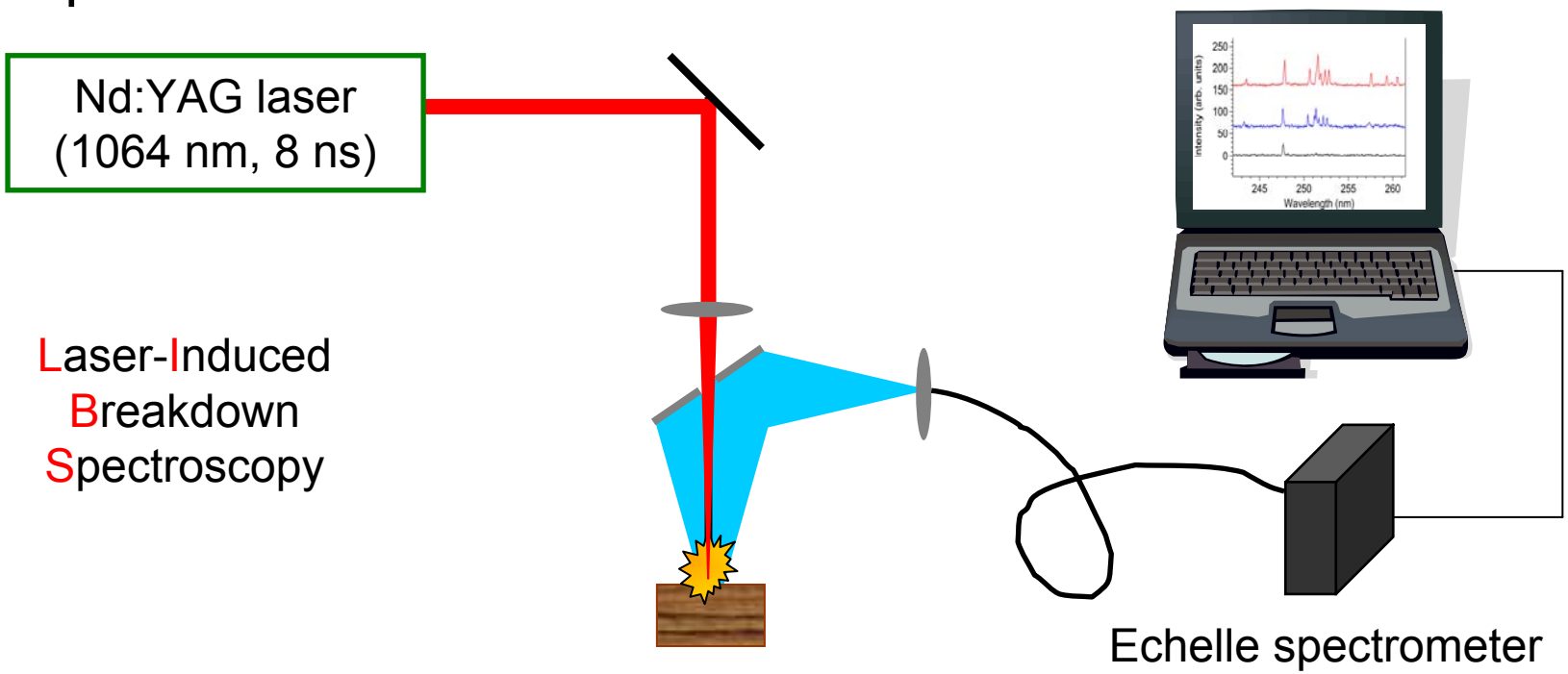
- genetic
- serological (antigenic)
- microbiological
- compositional
  - LIBS
  - Raman
  - MALDI-TOF-MS

things that make a LIBS-based technology powerful

- speed / portability / durability (ruggedness)
  - "rapid point-of-care diagnostic…"
- lack of complicated sample preparation
- no expertise required
- no genetic or antigenic precursors (consumables) necessary
- same technology / hardware useful for explosives, chemical, other threats (CBRNE capable)
- capability of sensor fusion

EMMA: Elemental Multivariate Microbiological Analysis

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

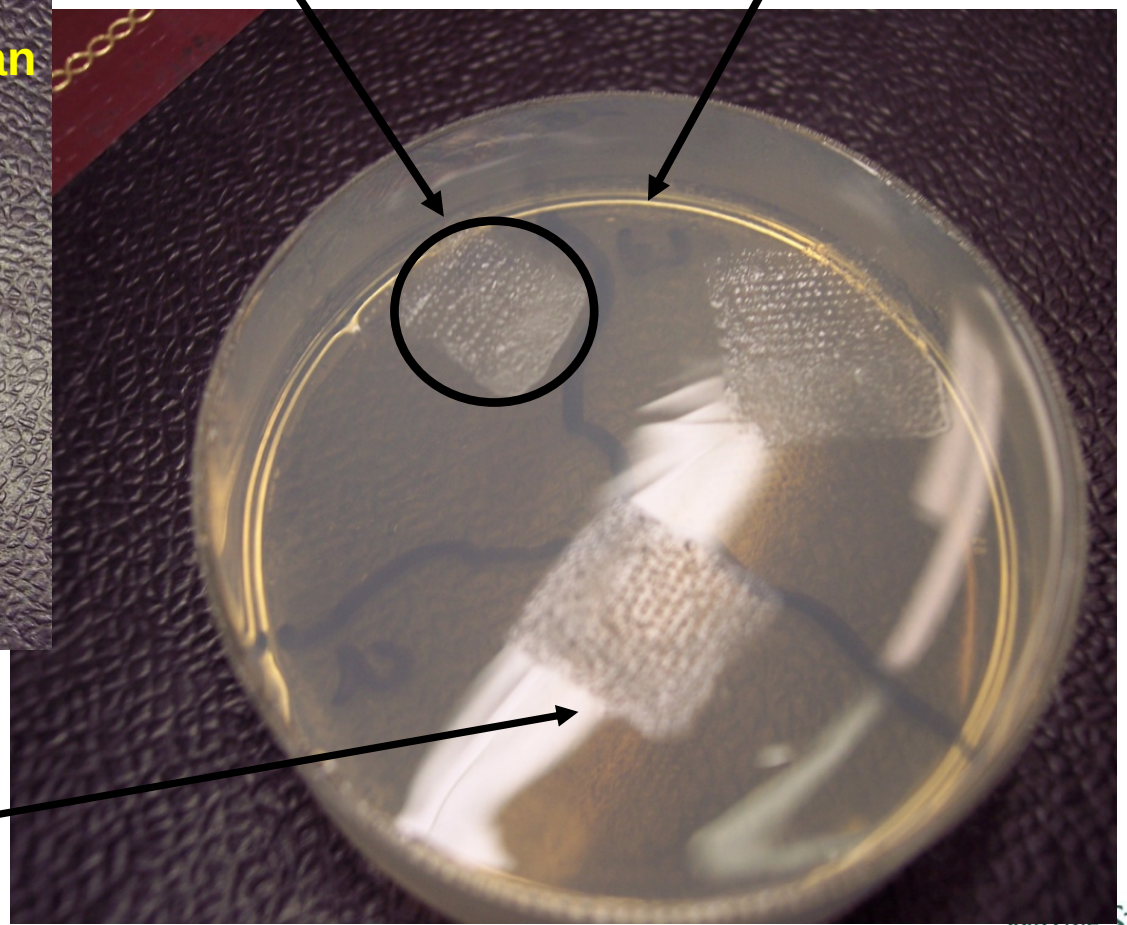


LIBS Spectrum is like a Spectral Fingerprint: Unique for Each Sample

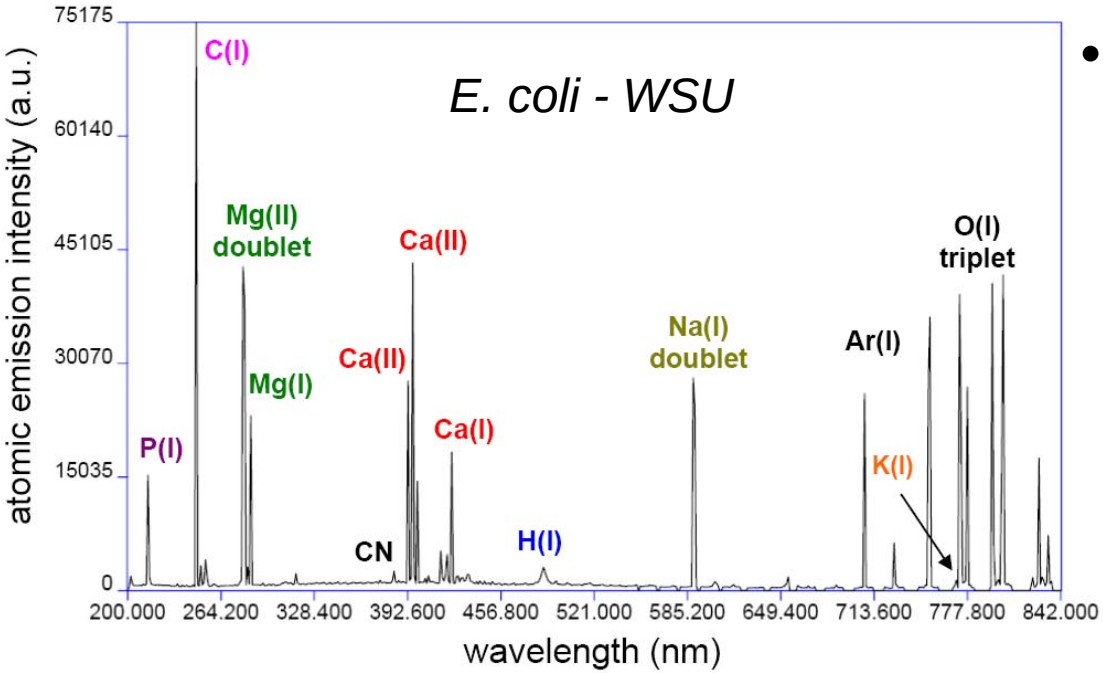
how we did it…

10 microliter of bacteria pellet

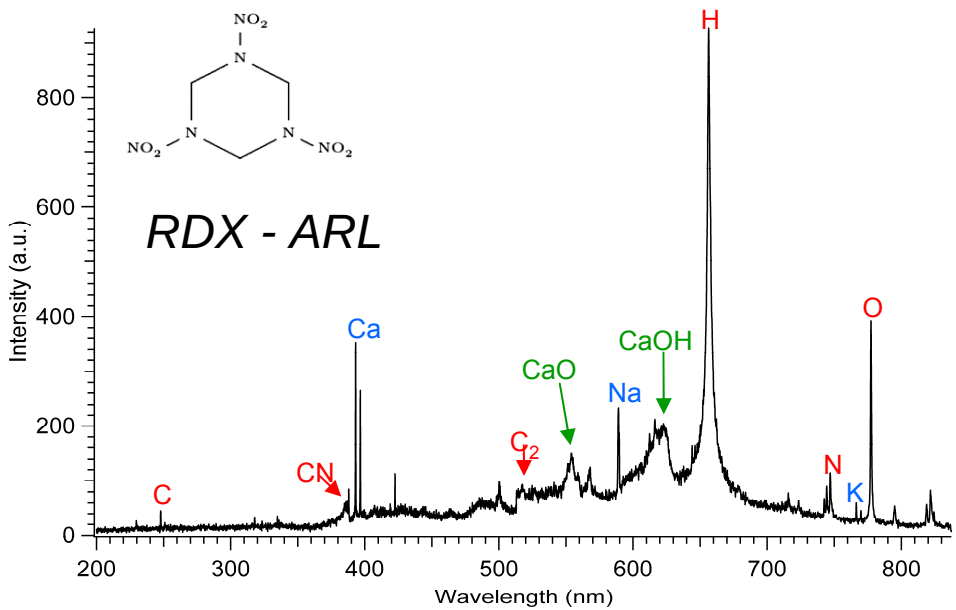
bacto-agar (99% water)



about 500-1500 bacteria per sampling location



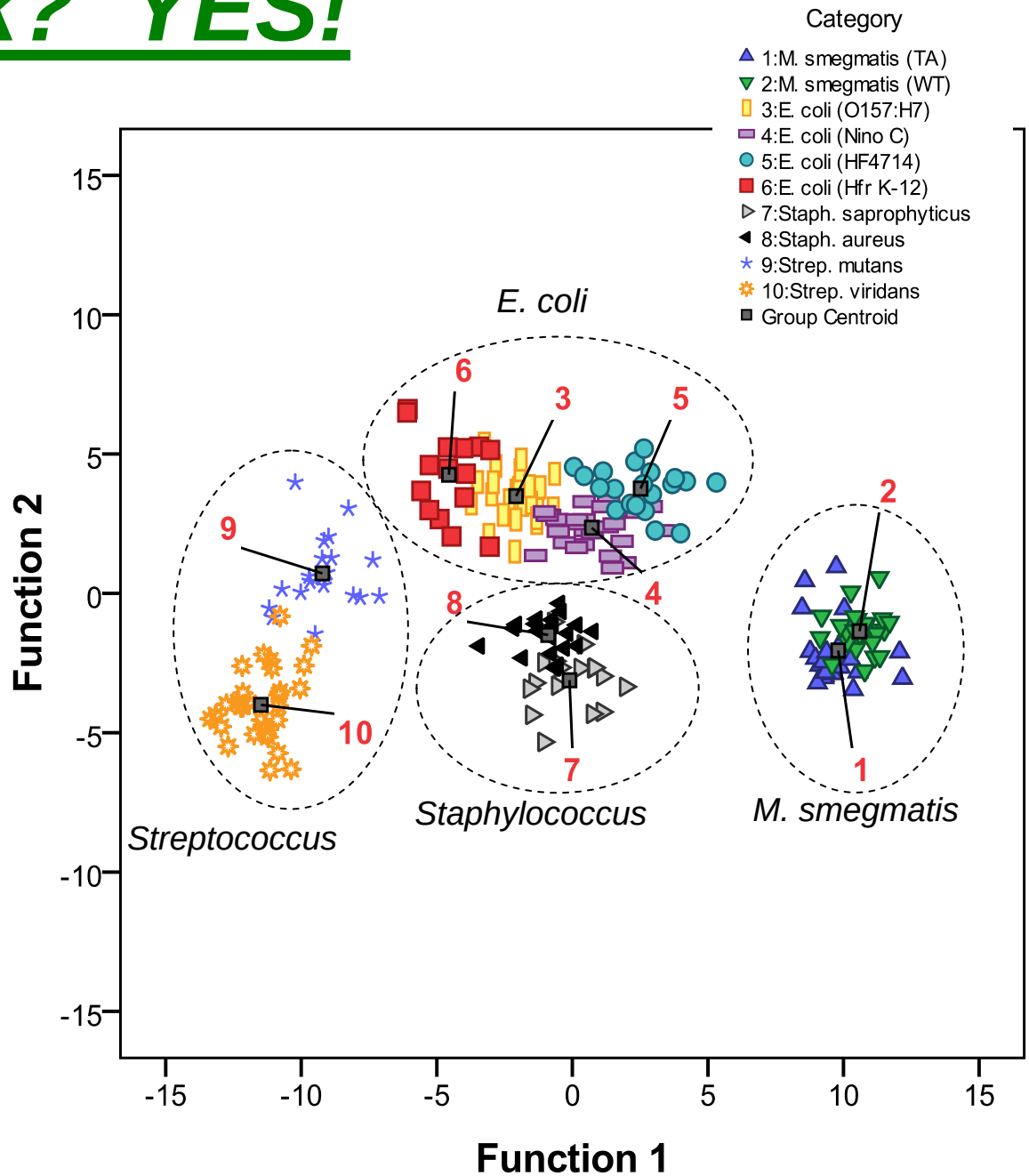
- high signal-to-noise atomic emission lines from inorganic elements allow a classification of the unknown target on the basis of its unique atomic spectrum



- concentrations of elements (or ratios of concentrations) become independent variables in a chemometric multivariate analysis (e.g. PCA, DFA, LDA, PLS-DA)

Does it work? YES!

- "Area under the curve" of 13 emission lines from 6 inorganic elements input as independent variables into a DFA.
- This test shows only the first two discriminant function scores for 10 different bacterial types (multiple genera, species, strains)



Group	Predicted Group Membership (%)									
	1	2	3	4	5	6	7	8	9	10
1:*M. smegmatis* (TA)	82.4	17.6	0	0	0	0	0	0	0	0
2:*M. smegmatis* (WT)	28.0	72.0	0	0	0	0	0	0	0	0
3:*E. coli* (O157:H7)	0	0	96.0	4.0	0	0	0	0	0	0
4:*E. coli* (C)	0	0	3.6	96.4	0	0	0	0	0	0
5:*E. coli* (HF4714)	0	0	0	0	100.0	0	0	0	0	0
6:*E. coli* (HfrK-12)	0	0	6.7	0	0	93.3	0	0	0	0
7:*Staph. saprophyticus*	0	0	0	0	0	0	94.1	5.9	0	0
8:*Staph. aureus*	0	0	0	0	0	0	0	100.0	0	0
9:*Strep. mutans*	0	0	0	0	0	0	0	0	95.0	5.0
10:*Strep. viridans*	0	0	0	0	0	0	0	0	0	100.0

The Wayne State Team has already demonstrated…

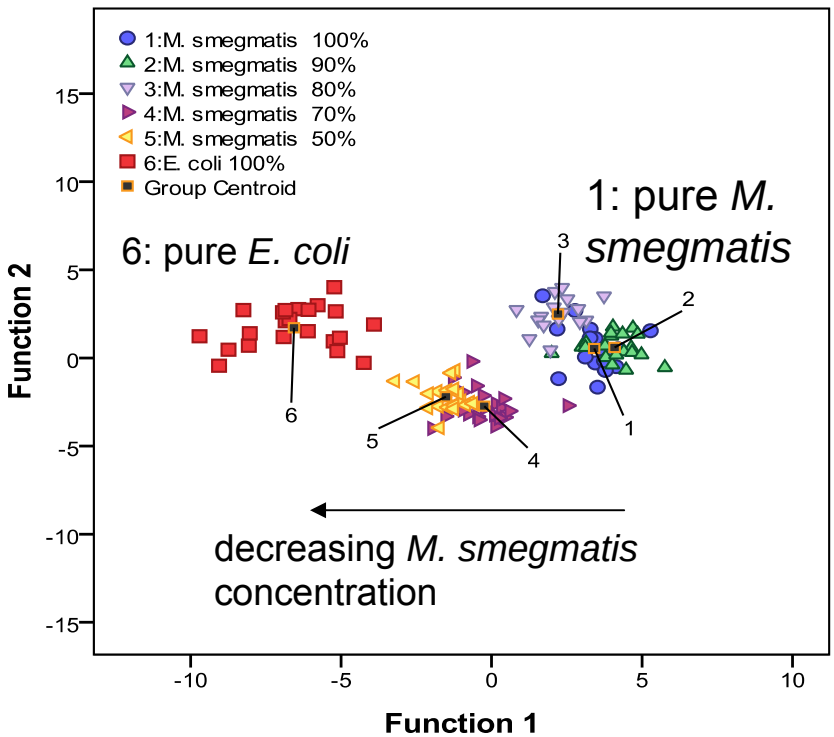
EMMA spectral fingerprint is:

- 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)
- capable of strain discrimination
- obtainable from about 500 bacteria

6 publications in Applied Physics Letters, Journal of Applied Physics, Applied Optics, and Spectrochimica Acta B

"Mixed" Samples

Category	# of Spectra	Classification Results		
		M. smegmatis	*E. coli*	*S. viridans*
100% *M. smegmatis*, 0% *E. coli*	21	100%	0%	0%
90% *M. smegmatis*, 10% *E. coli*	20	100%	0%	0%
80% *M. smegmatis*, 20% *E. coli*	16	100%	0%	0%
70% *M. smegmatis*, 40% *E. coli*	21	76%	24%	0%
50% *M. smegmatis*, 50% *E. coli*	19	47%	53%	0%
0% *M. smegmatis*, 100% *E. coli*	25	0%	100%	0%



- Six separate mixtures of known mixing fraction were prepared from suspensions *M. smegmatis* and *E. coli* C.
- As long as the majority bacterium comprised 80% of the mixture, we saw 100% identification.

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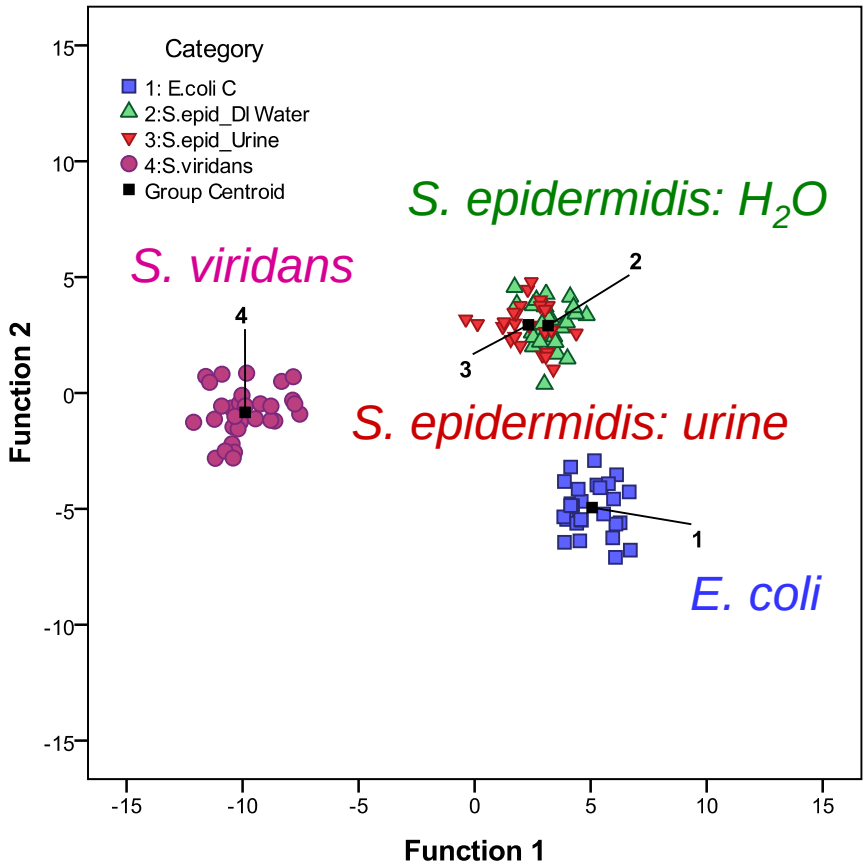
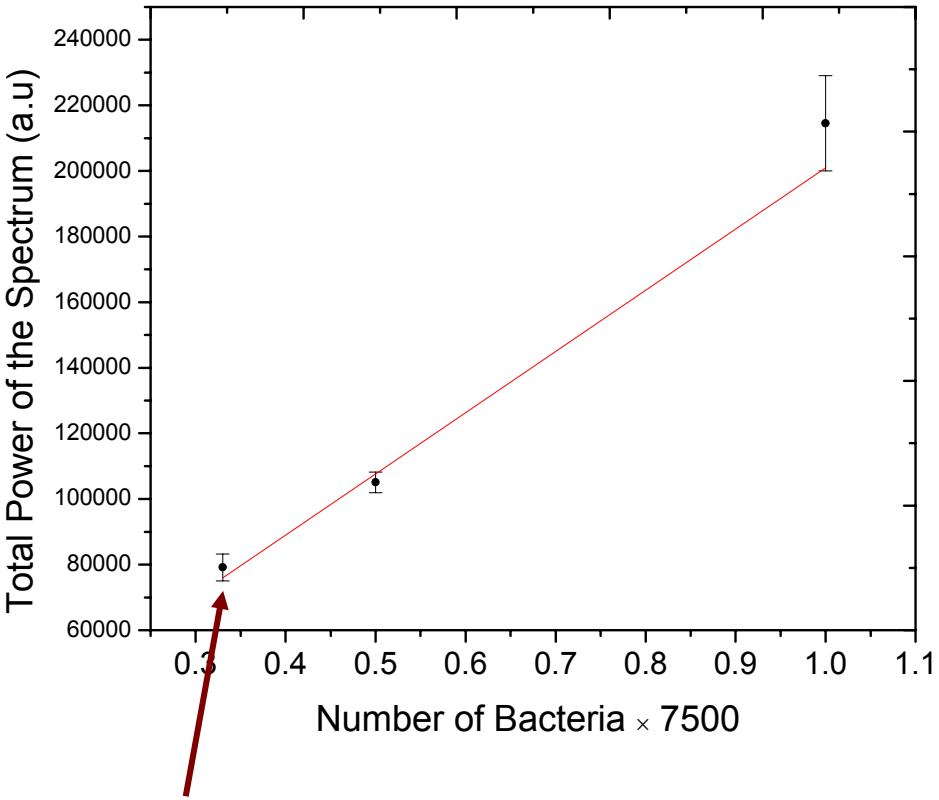
“Dirty” samples



- Samples of *Staph. epidermidis* were prepared in DI water and sterile urine.
- Samples were collected and tested via LIBS with NO WASHING.
- LIBS spectral fingerprint from urine-exposed bacteria were identical to water-exposed bacteria.
- EMMA correctly classified 100% of the urine-exposed bacteria as being consistent with *S. epidermidis*

LIBS intensity linearly dependent on number of bacteria



5 laser sampling locations

~500 bacteria per locations

- Samples of *E. coli* with different titer tested on agar.
- Each data point is the average of 5 sampling locations.
- As expected, spectra demonstrate a linear dependence with cell number.
- All spectra were 100% correctly identified (specificity not dependent on number of cells).
- Suggests an antibiotic resistance test?

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Strain discrimination confirmed by others…

The Use of Laser-Induced Breakdown Spectroscopy for Distinguishing Between Bacterial Pathogen Species and Strains

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Applied Research Associates, Inc., 4300 San Mateo Blvd NE Suite A-220, Albuquerque, New Mexico 87110 (R.A.M., D.A.C.); and Department of Biology, New Mexico State University, P.O. Box 30001, Las Cruces, New Mexico, 88003-8001 (J.M.D., J.G.)

APPLIED SPECTROSCOPY Volume 64, Number 7, 2010

- 100% accuracy exhibited in blind trials of 4 MRSA strains and one *E. coli* strain
- lyophilized ("freeze-dried") specimens used

We Must Proceed, and Faster…

LIBS 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

what must we do to make LIBS a clinical tool?

Develop protocols for clinical sample preparation (blood, urine, sputum)

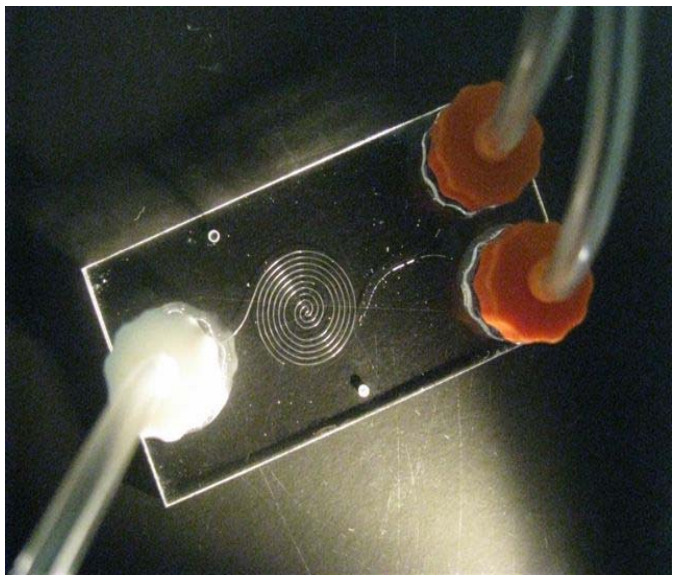
- isolation
- concentration under the laser focus

solutions

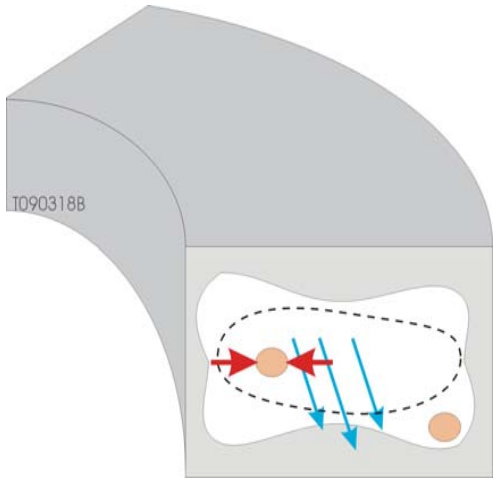
1. differential centrifugation
2. filtration (sequential?)
3. optical trapping / separation
4. microfluidic separation
5. antibody isolation/phage display technology (consumables!)

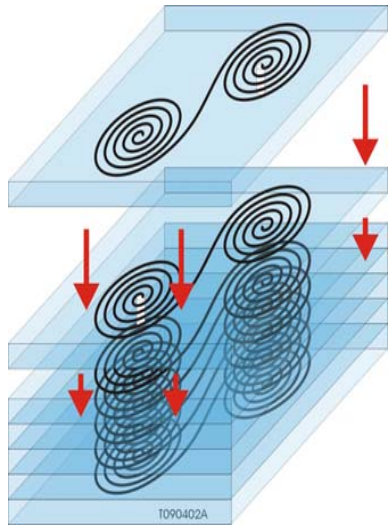
Microfluidic separation/concentration

(Translume, Inc. Ann Arbor, MI)



monolithically fabricated devices in glass

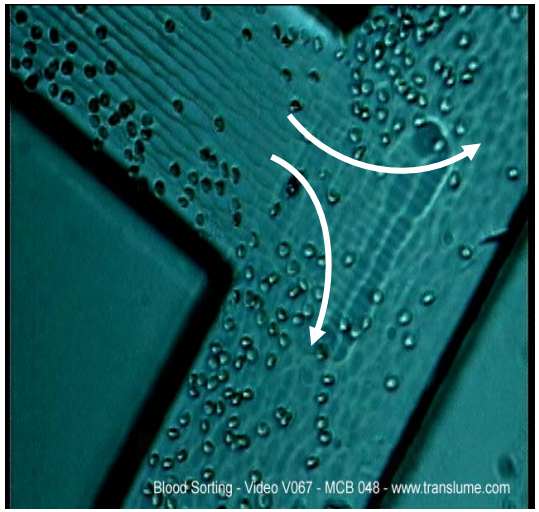


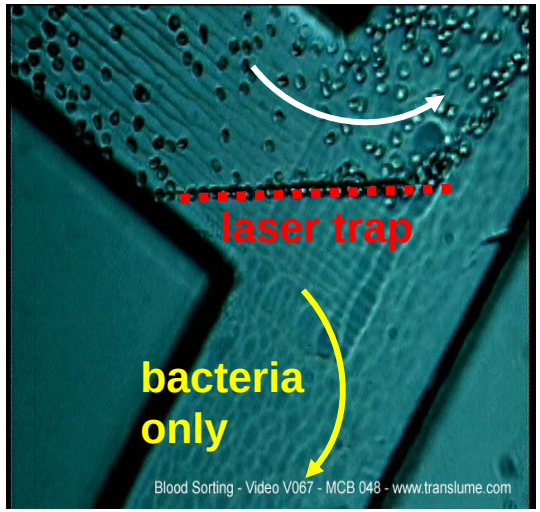


hydrodynamic (microfluidic) separation of heavier cells from lighter cells

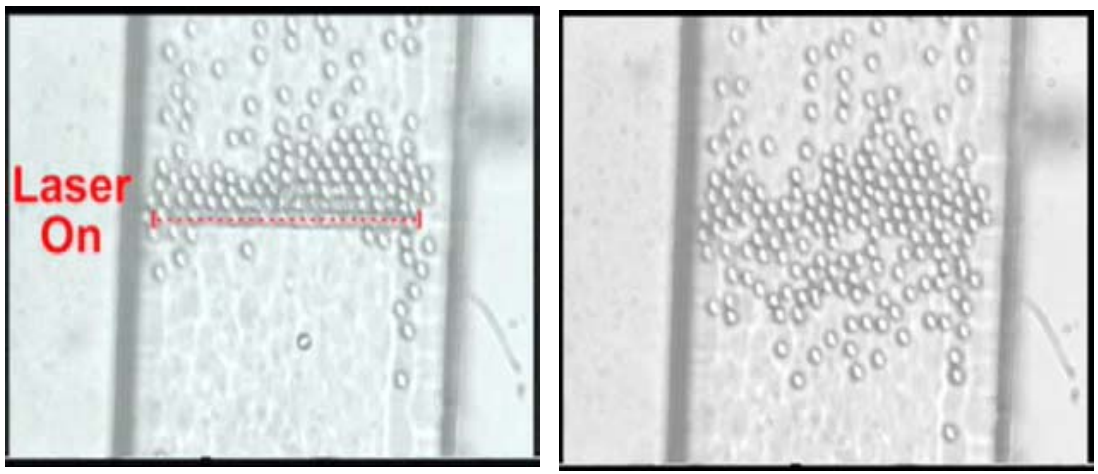
Microfluidic separation/concentration

(Translume, Inc. Ann Arbor, MI)

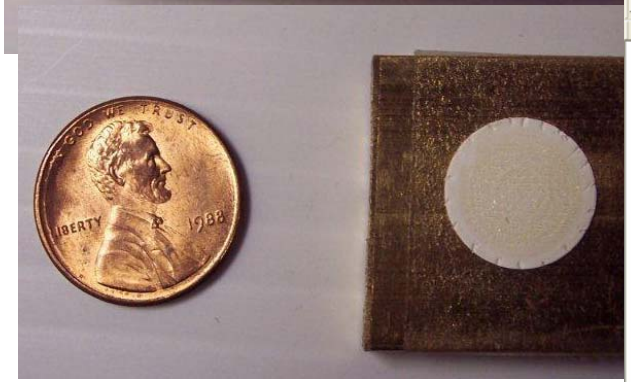




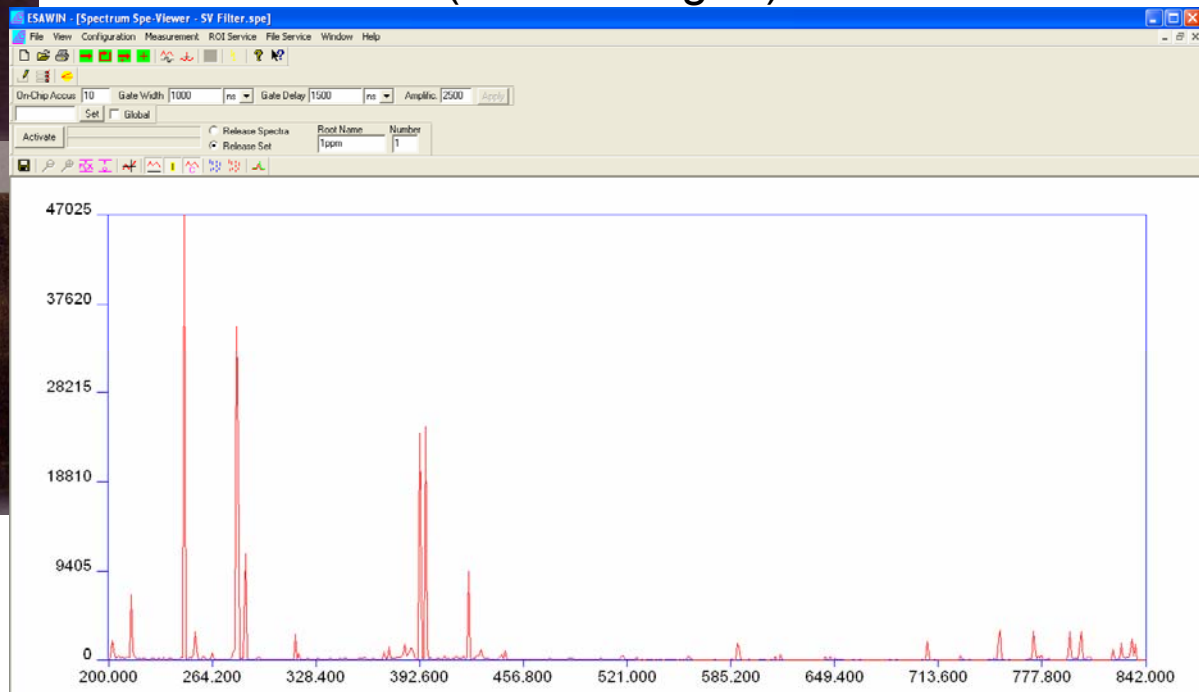
optical trap-based separation of heavier cells from lighter cells



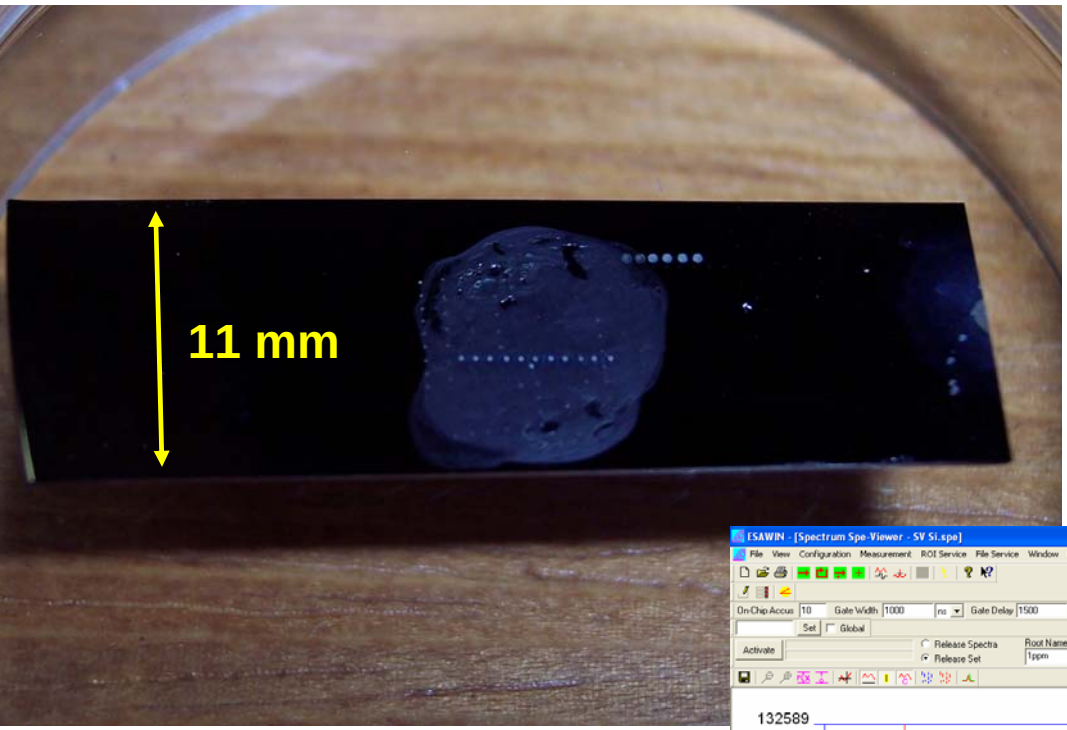
Novel substrates 1



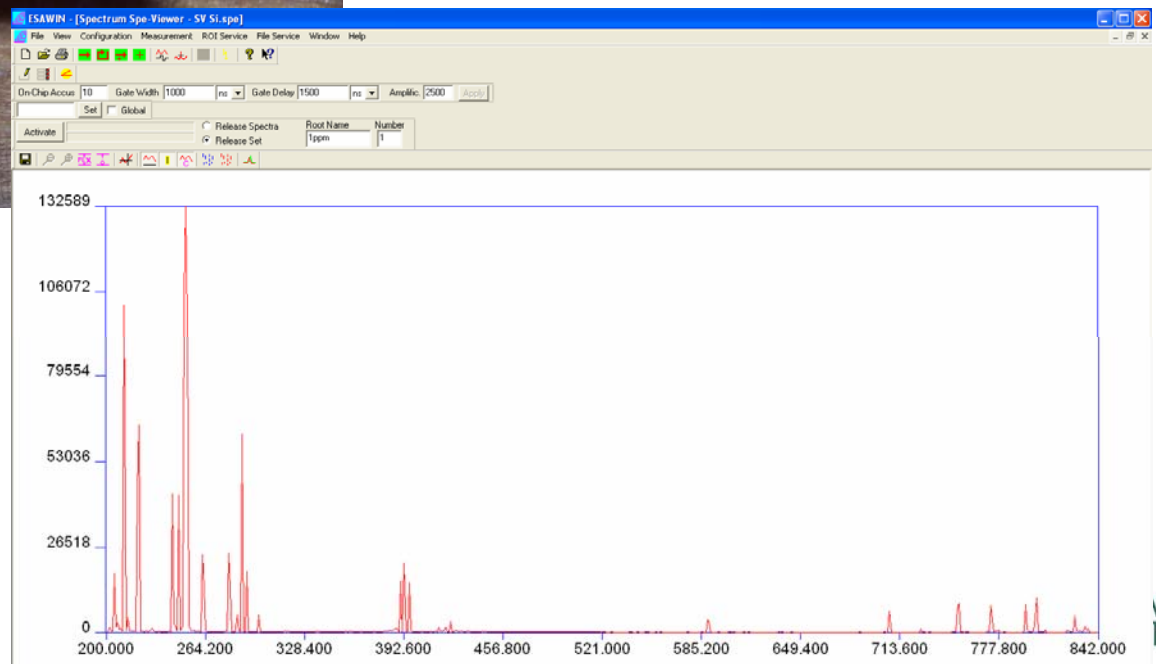
- 10 mL of a suspended bacterial culture pushed through a 0.22 or 0.44 µm cellulose (carbon) Millipore filter
- alternately, bacteria just deposited on filter (wicking)
- C line does “contaminate” spectrum, but only at 7% level (same as agar!)



Novel substrates 2



- Acid etched “porous” silicon
- Bacteria fixed with polyacrimide
- High SNR LIBS spectrum
- Si lies do not contaminate spectrum

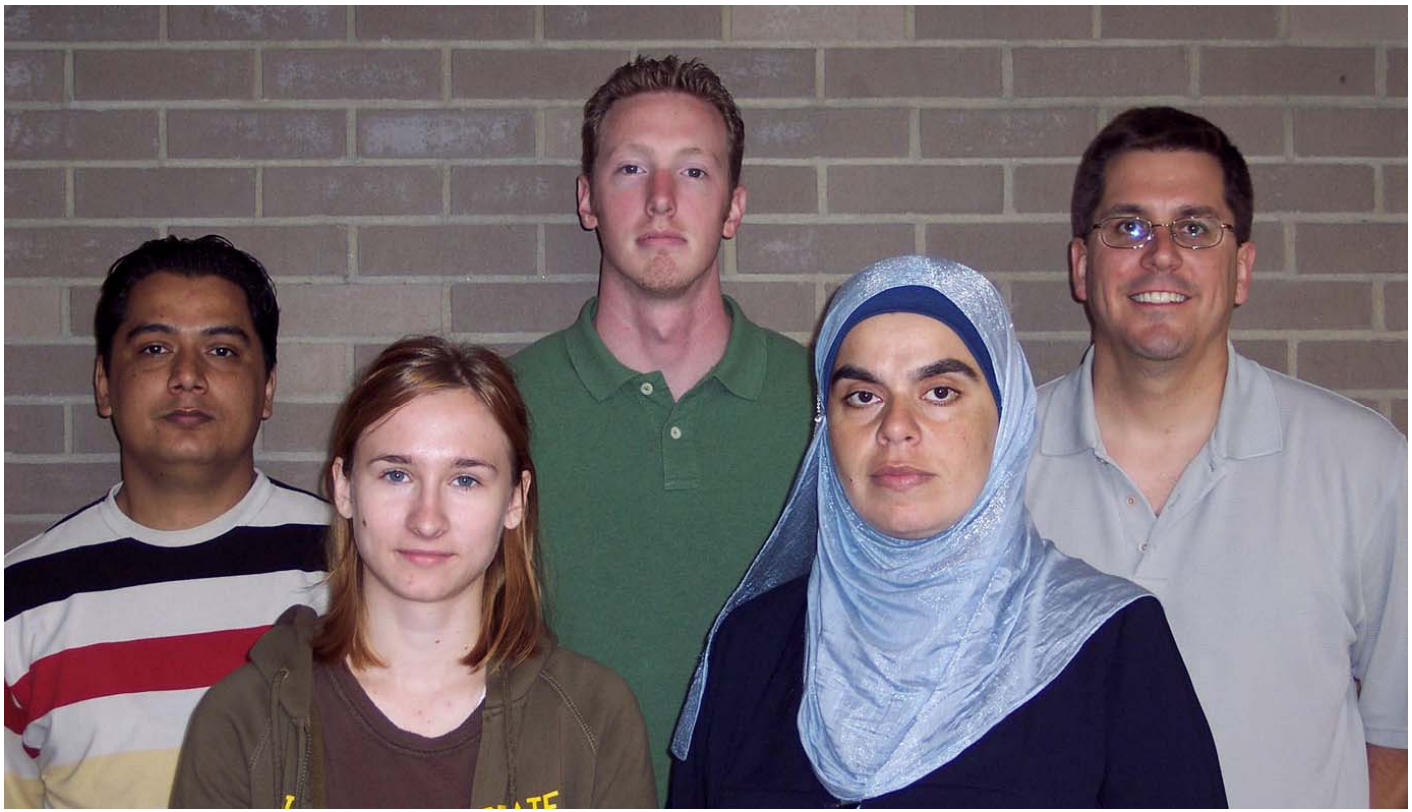


Conclusions

- All EMMA experiments to date have successfully shown the utility of LIBS to identify bacterial samples in a variety of growth conditions, in mixed samples, in dirty samples, etc.

- We are ready to move to testing real “clinical” type samples through our in-place organizational structure, which combines expertise in hardware development, software development, microbiological handling, and LIBS development.

My students



Thank you!

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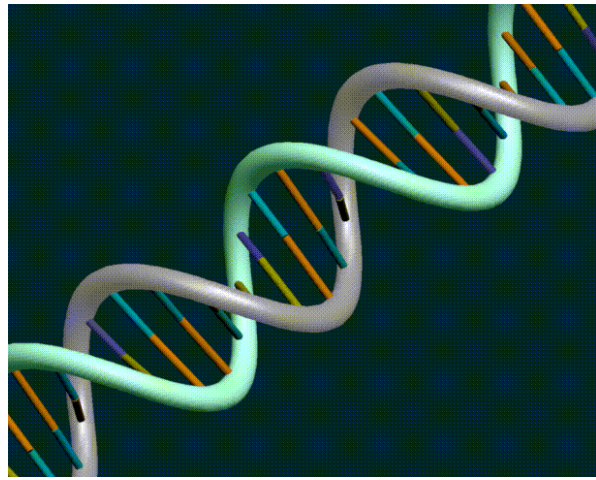
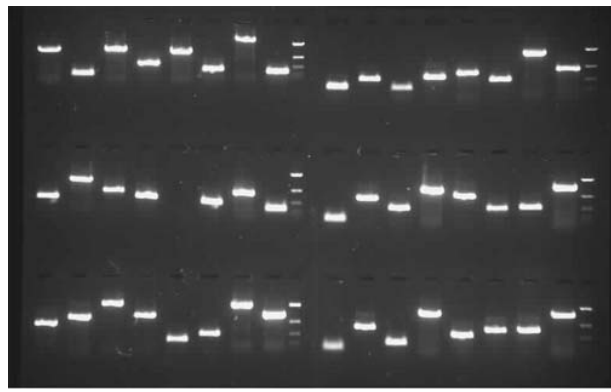
genetic



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



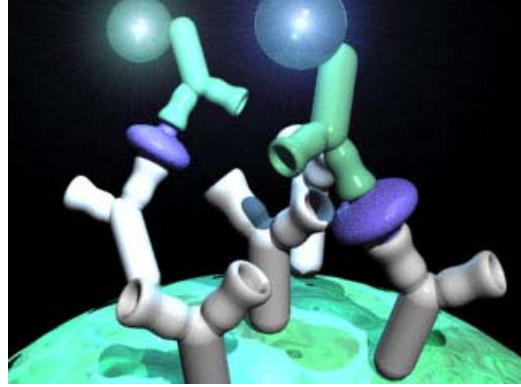
requires

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

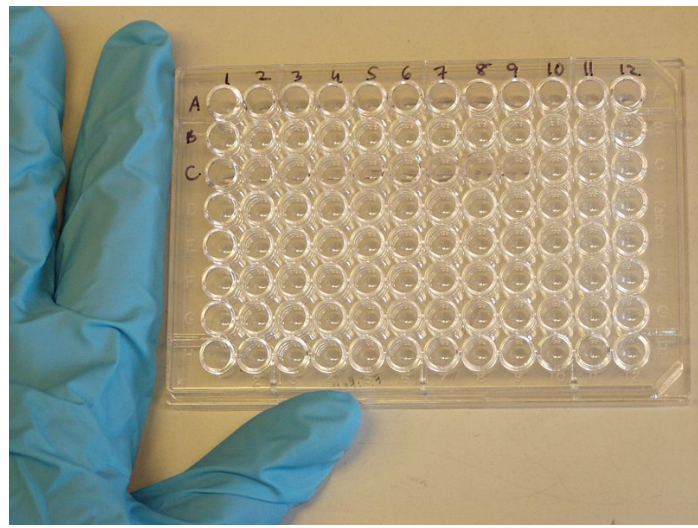
drawbacks

- 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

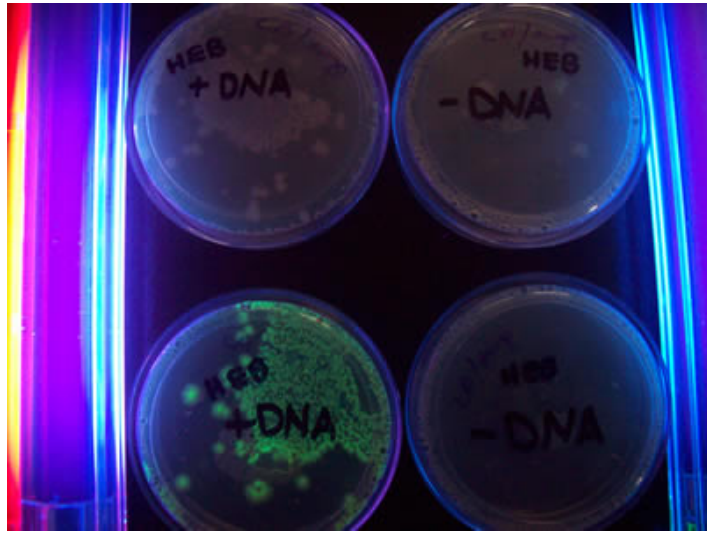
- *a priori* knowledge of serology (surface antigens)

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