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

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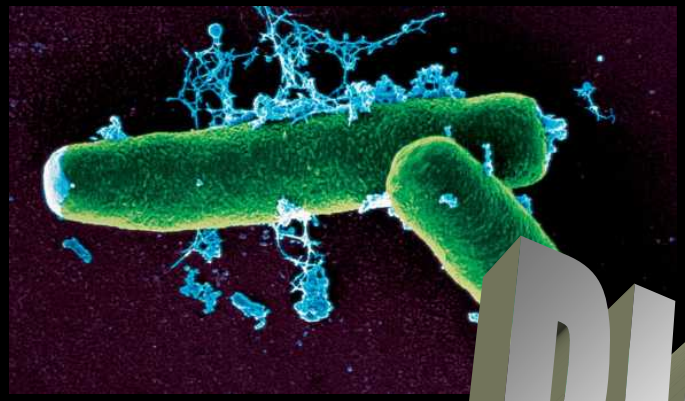
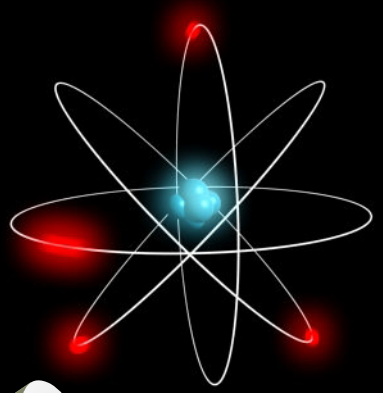
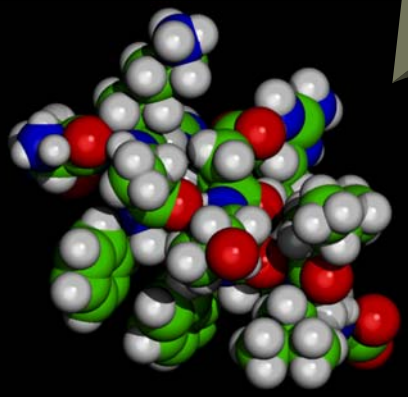
The BIOMAS Project:

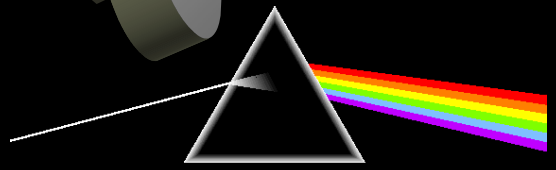
Bacterial Identification by Optical, Molecular, and Atomic Spectroscopy

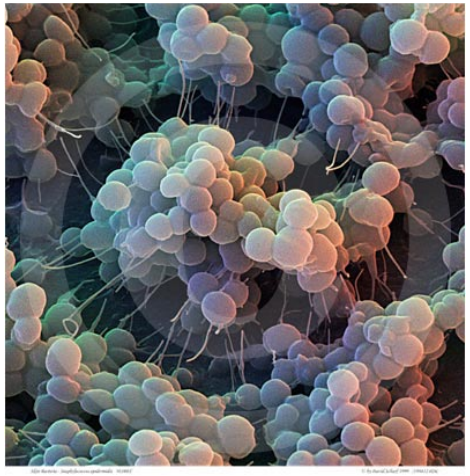




BIOMAS

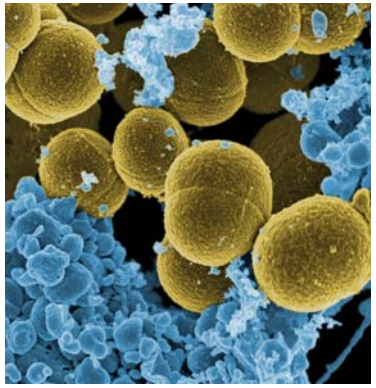






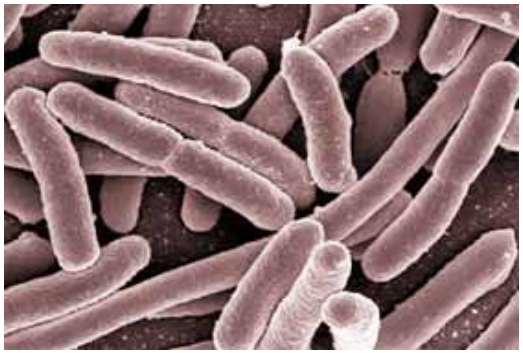
Staph. epidermidis

Staph. aureus

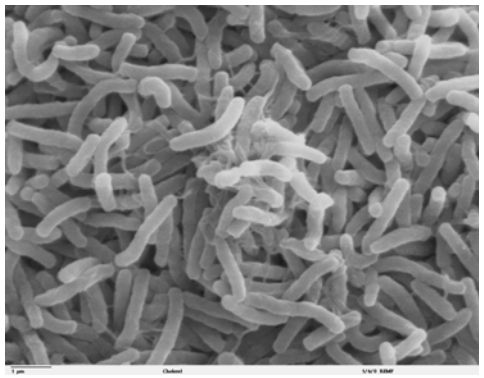


there is an urgent need right now in the military, civilian (hospital, food processing, environmental), and first responder communities for a "…rapid point-of-care (multiplex?) diagnostic for disease-causing pathogens."

E. coli



V. cholerae

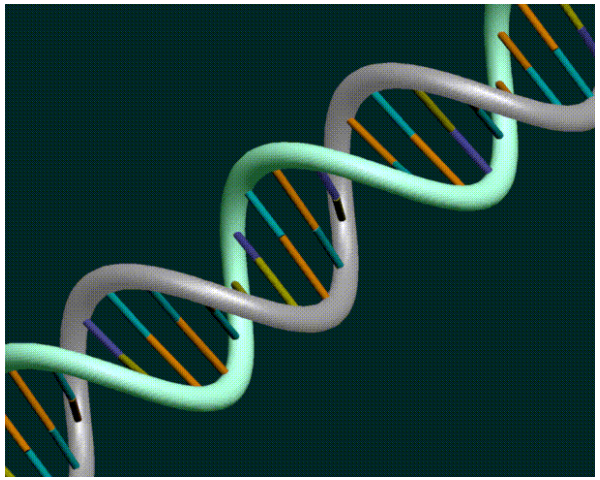


How do we identify bacteria?

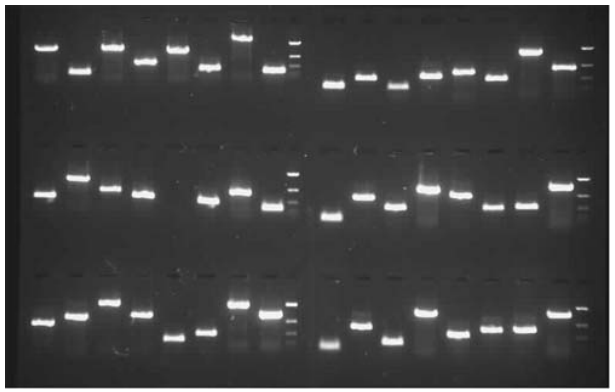
4 ways

- genetic
- serological (antigenic)
- microbiological
- compositional (LIBS)

genetic



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



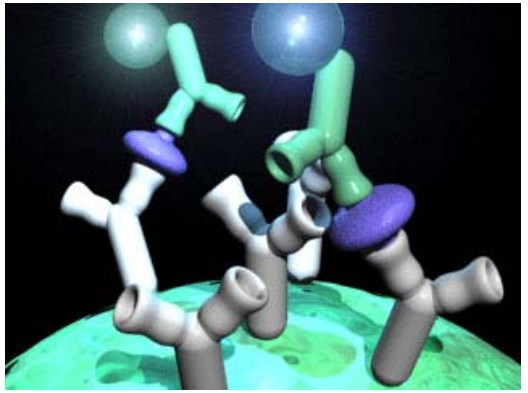
requires

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

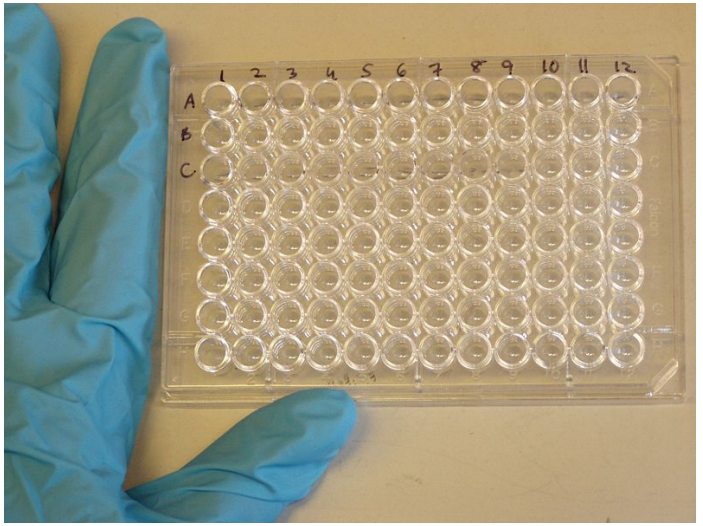
drawbacks

- 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

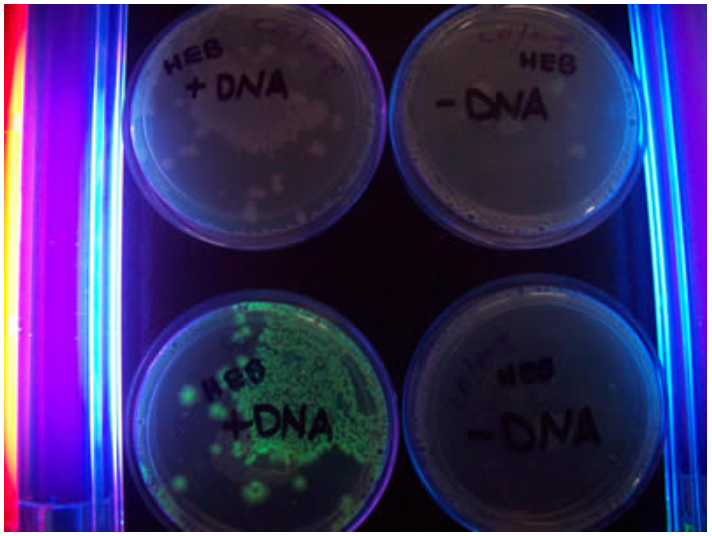
- *a priori* knowledge of serology (surface antigens)

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

- Raman
- Mass-spectrometry
- **LIBS**

requires

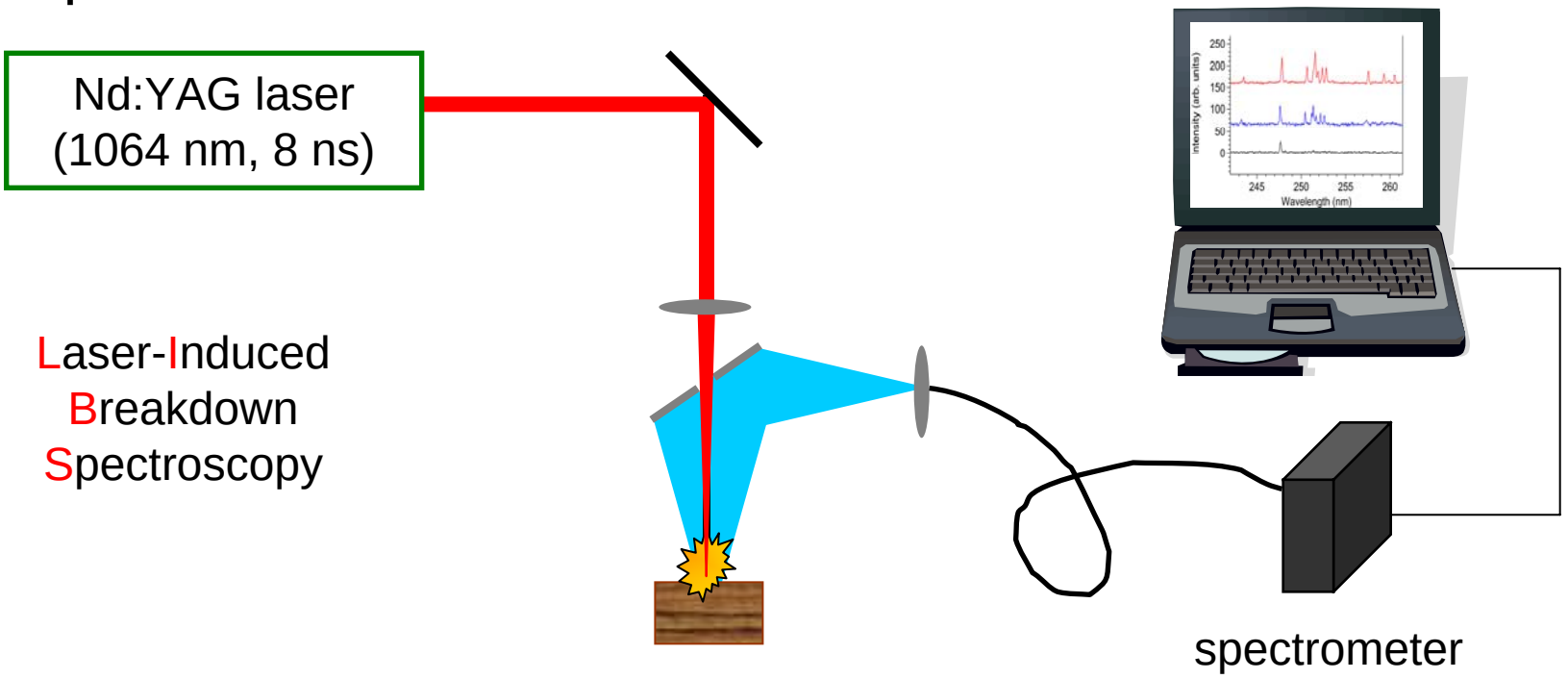
- no *a priori* knowledge of serology (surface antigens)
- no *a priori* knowledge of genetic sequence
- no consumables (hopefully)
- no expertise

drawbacks

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

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

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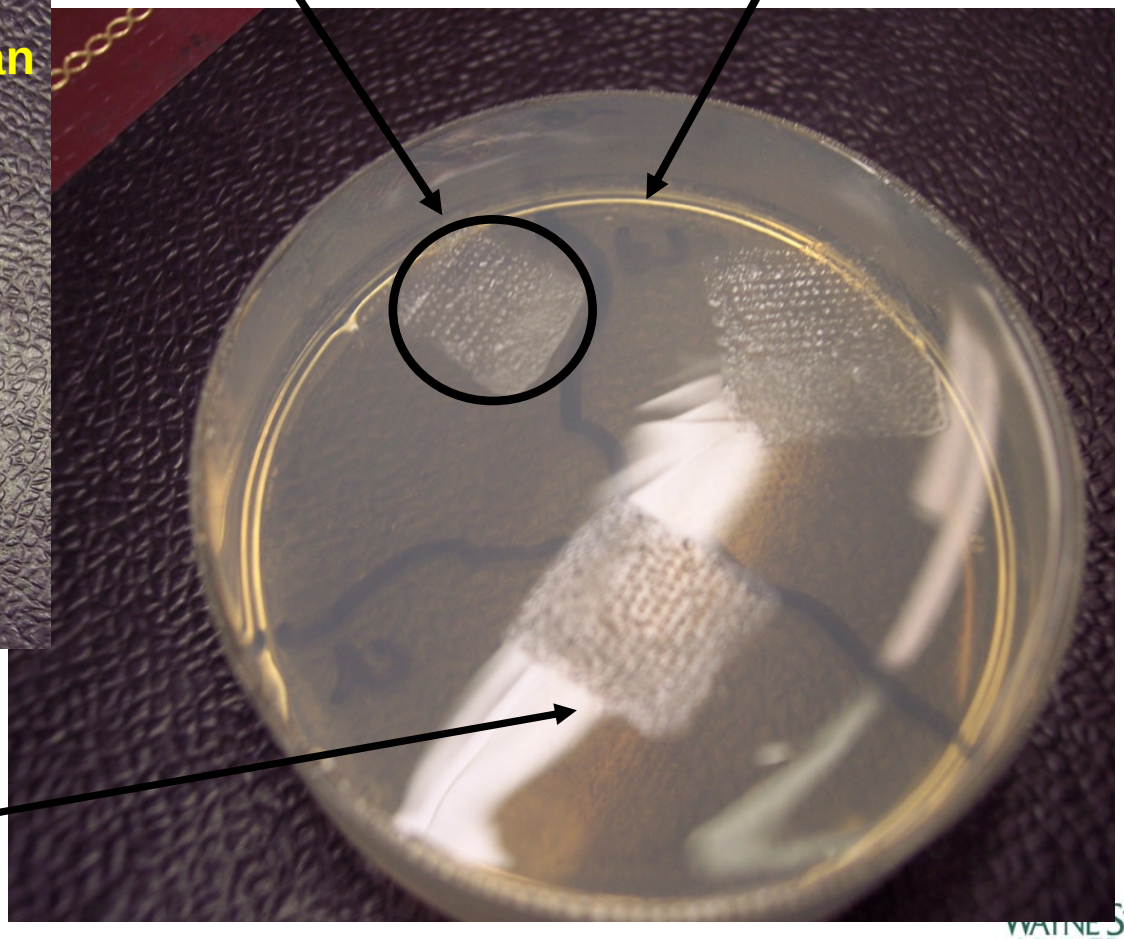
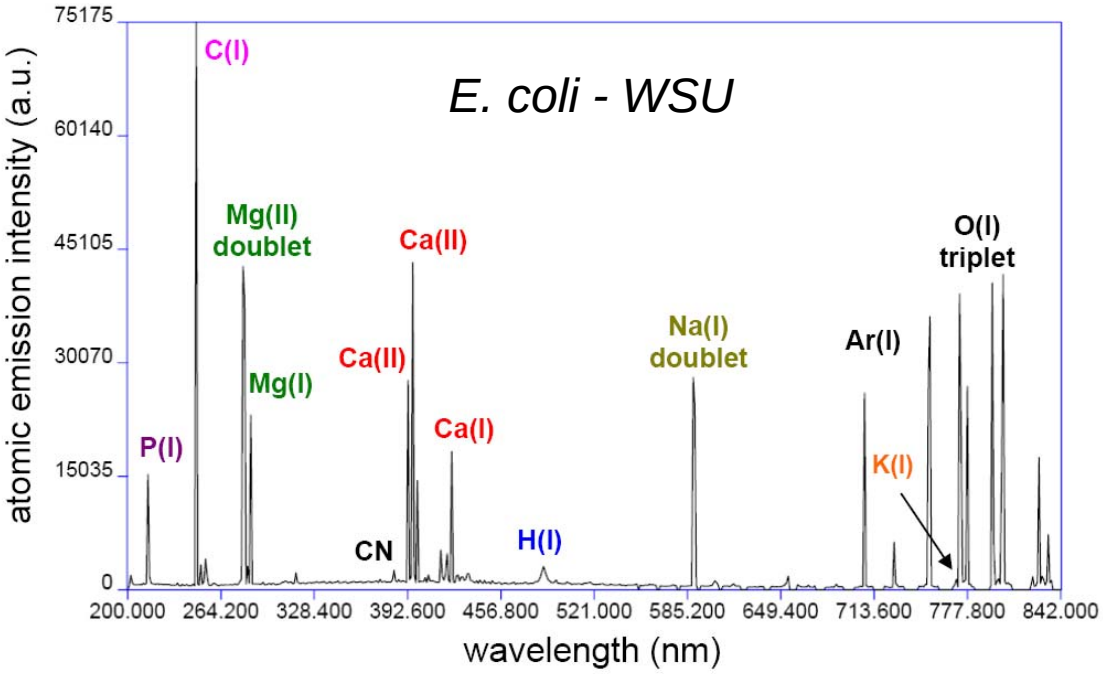
how we did it…

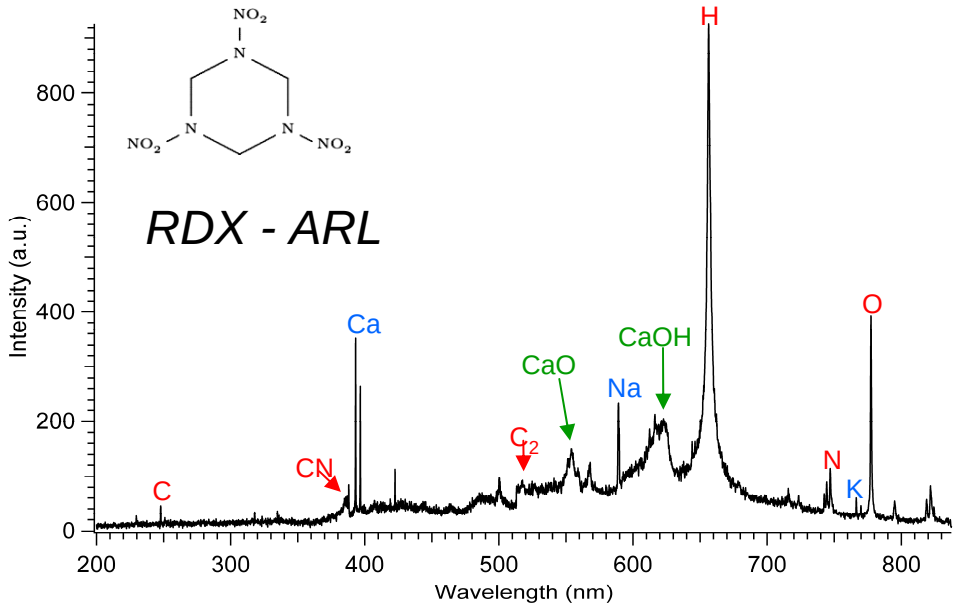
10 microliter of bacteria pellet

bacto-agar (99% water)



about 500-1500 bacteria per sampling location





- advanced signal-processing statistical techniques (“chemometrics”) classify/identify the unknown target on the basis of its unique atomic signature
- concentrations of elements (or ratios of concentrations) become independent variables in a chemometric multivariate analysis

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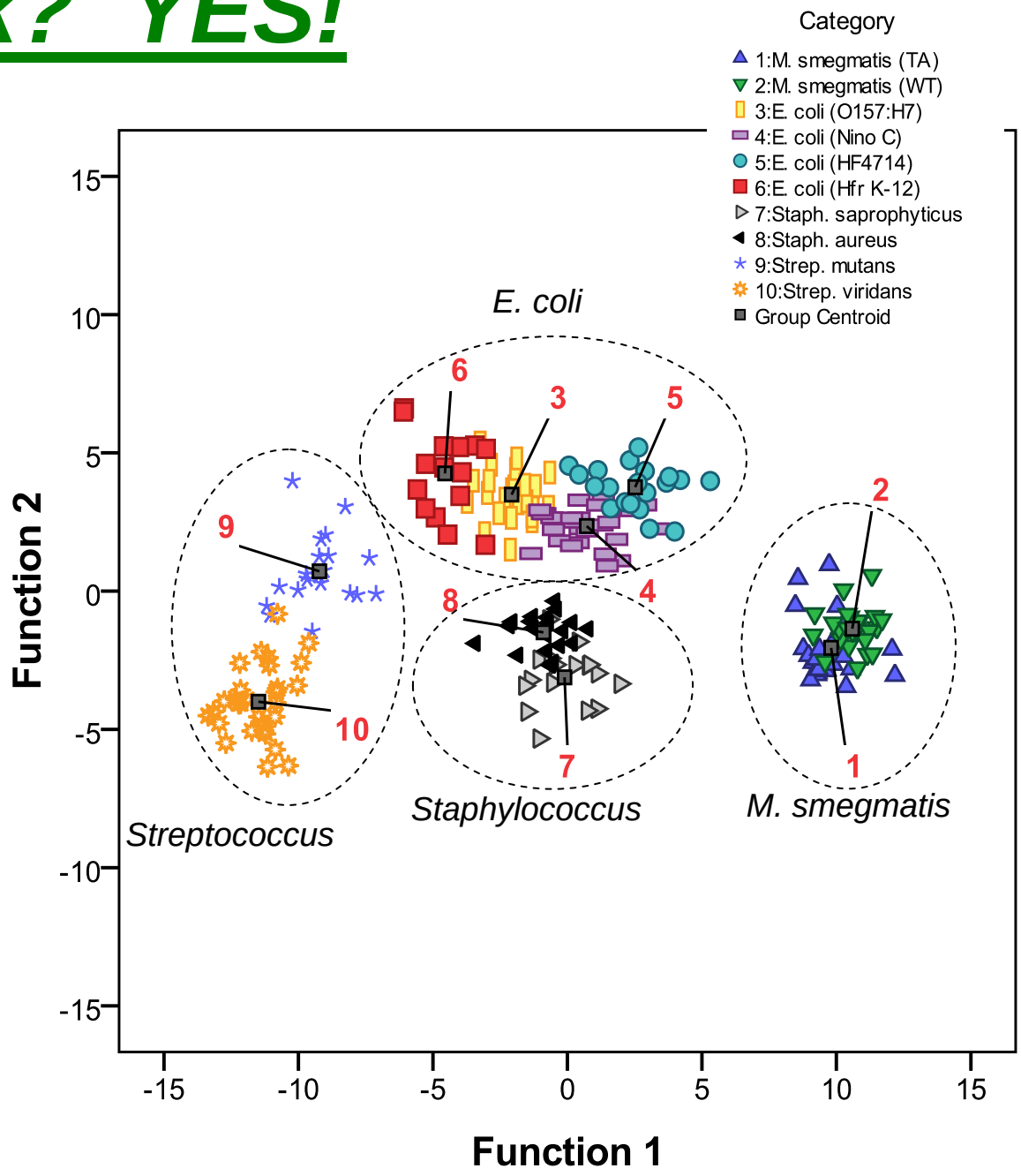
things that make EMMA technology unique

- 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

Does it work? YES!

- Intensity of lines, ratios of intensities used in a statistical multi-variate analysis

- Discriminant function analysis (DFA)
  - principal component analysis (PCA)
  - partial least squares – discriminant analysis (PLS-DA)
  - linear discriminant analysis (LDA)



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

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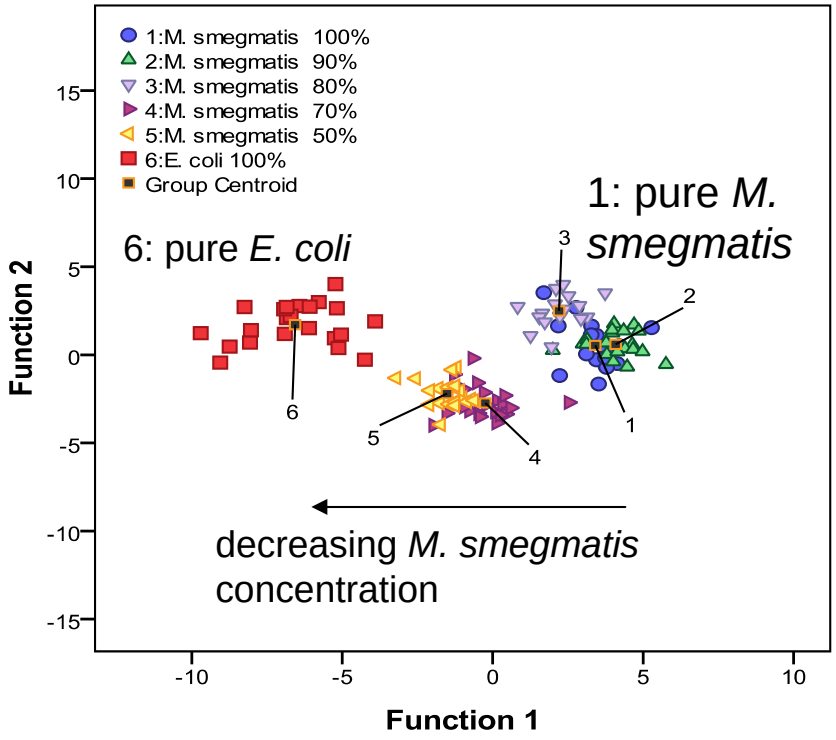
"Mixed" Samples

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%



- Mixtures of known mixing fraction were prepared from suspensions *M. smegmatis* and *E. coli* C.
- Six separate mixtures were prepared with a ratio *M. smegmatis* to *E. coli* C given by M_{1-x}:C_x with x = 0.0, 0.1, 0.2, 0.3, 0.5, 1.0.
- Multiple 1.5 mL tubes of these mixtures were prepared, thoroughly agitated via vortex mixing, then centrifuged for 3 minutes at 5000 rev/min.

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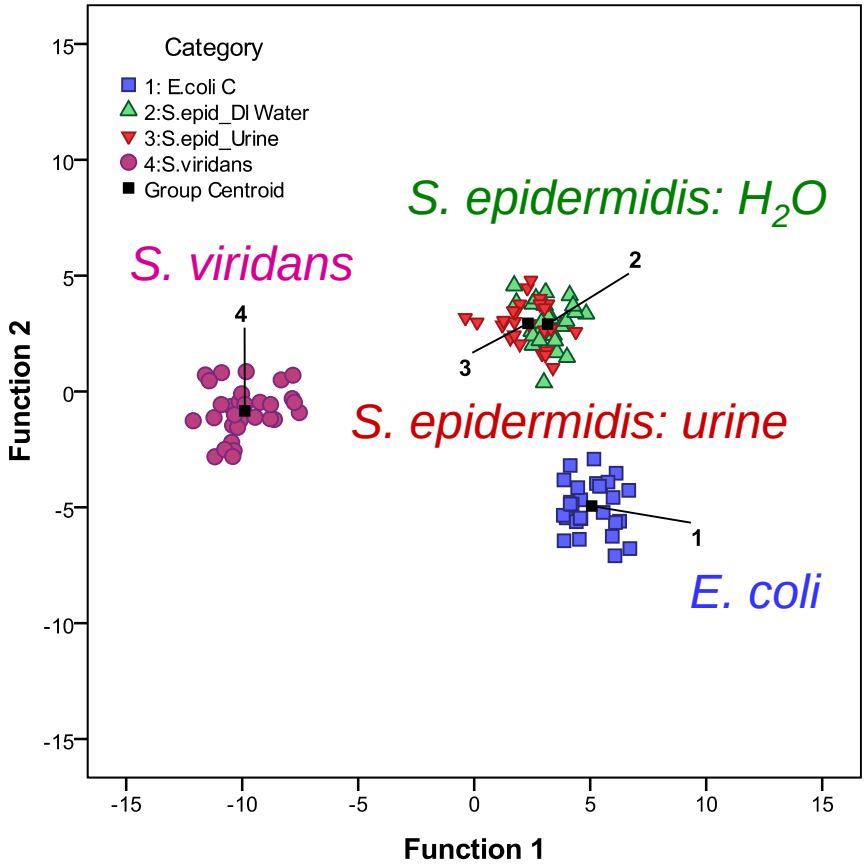
“Dirty” samples



- 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*

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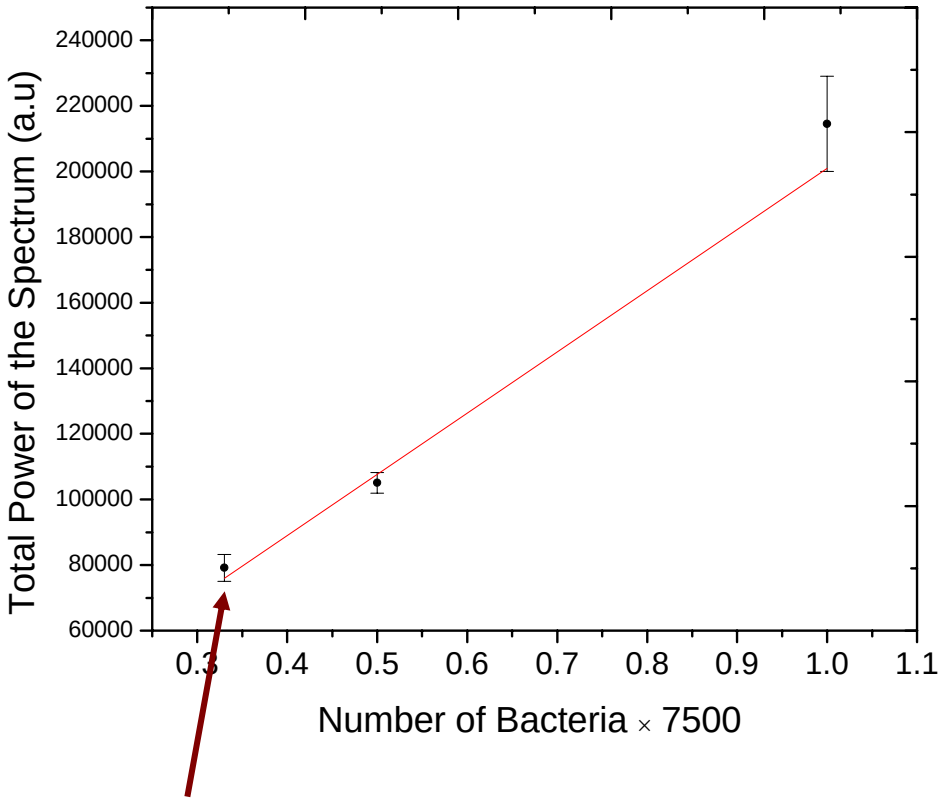
LIBS intensity linearly dependent on number of bacteria



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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what must we do to make LIBS a clinical tool?

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

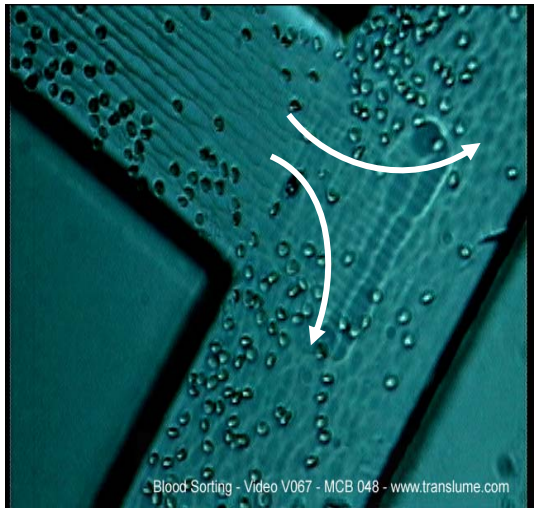
- isolation
- concentration under the laser focus

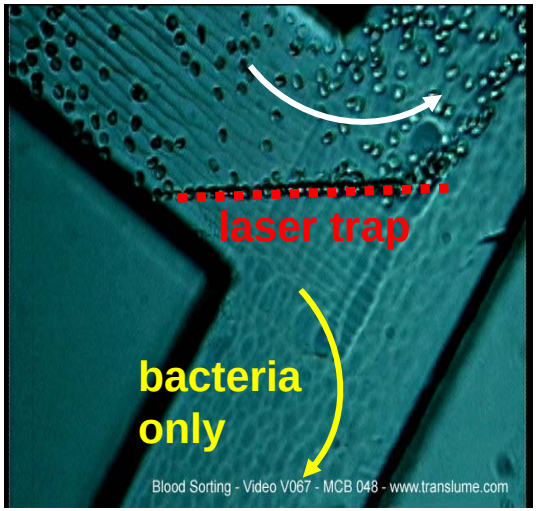
solutions

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

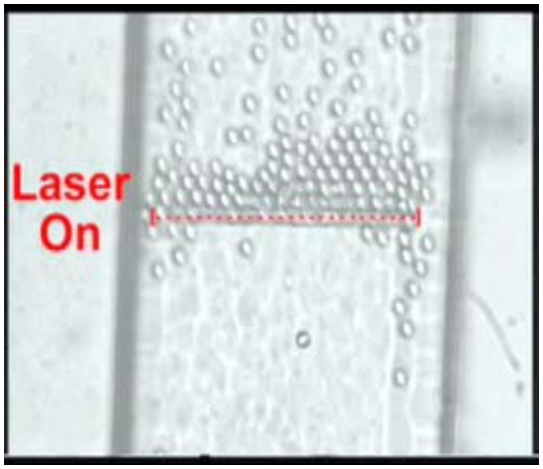
Microfluidic separation/concentration

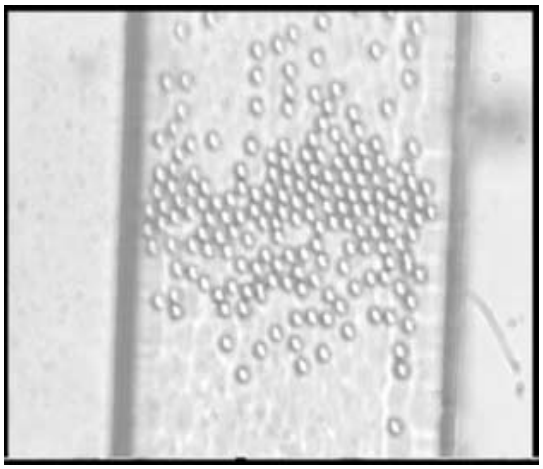
(Translume, Inc. Ann Arbor, MI)





optical trap-based separation of heavier cells from lighter cells





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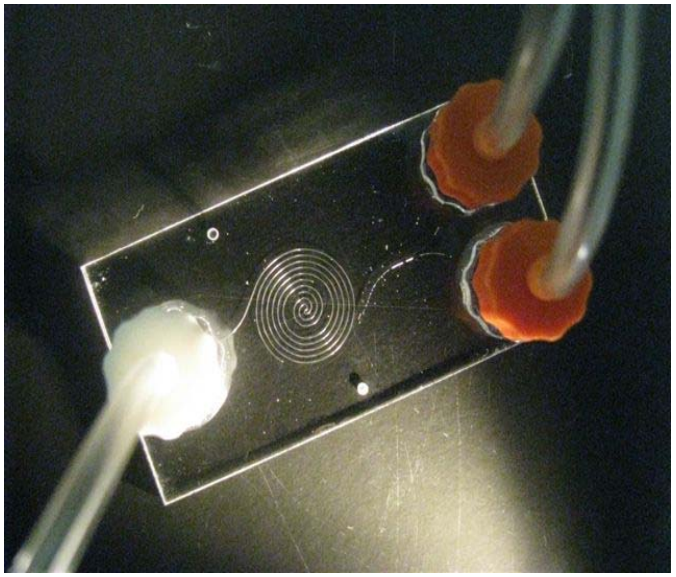
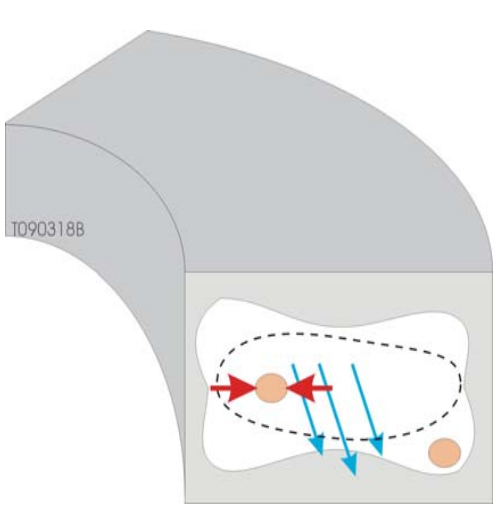
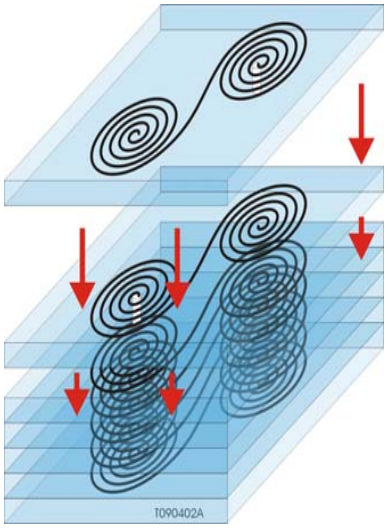
Microfluidic separation/concentration

(Translume, Inc. Ann Arbor, MI)



monolithically fabricated devices in glass

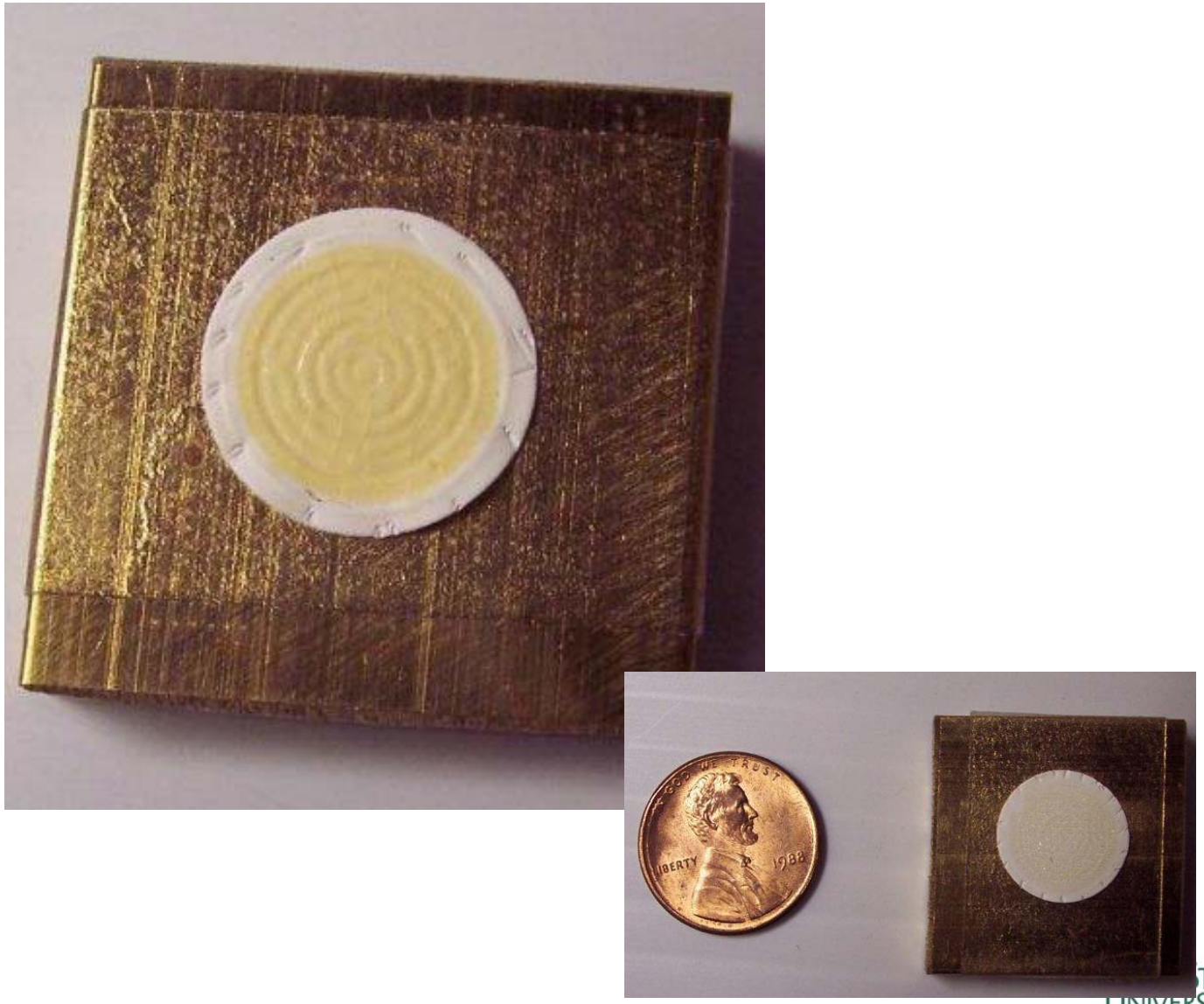




hydrodynamic (microfluidic) separation of heavier cells from lighter cells

Filtration

10 mL of a suspended bacterial culture pushed through a 0.22 μm cellulose (carbon) Millipore filter



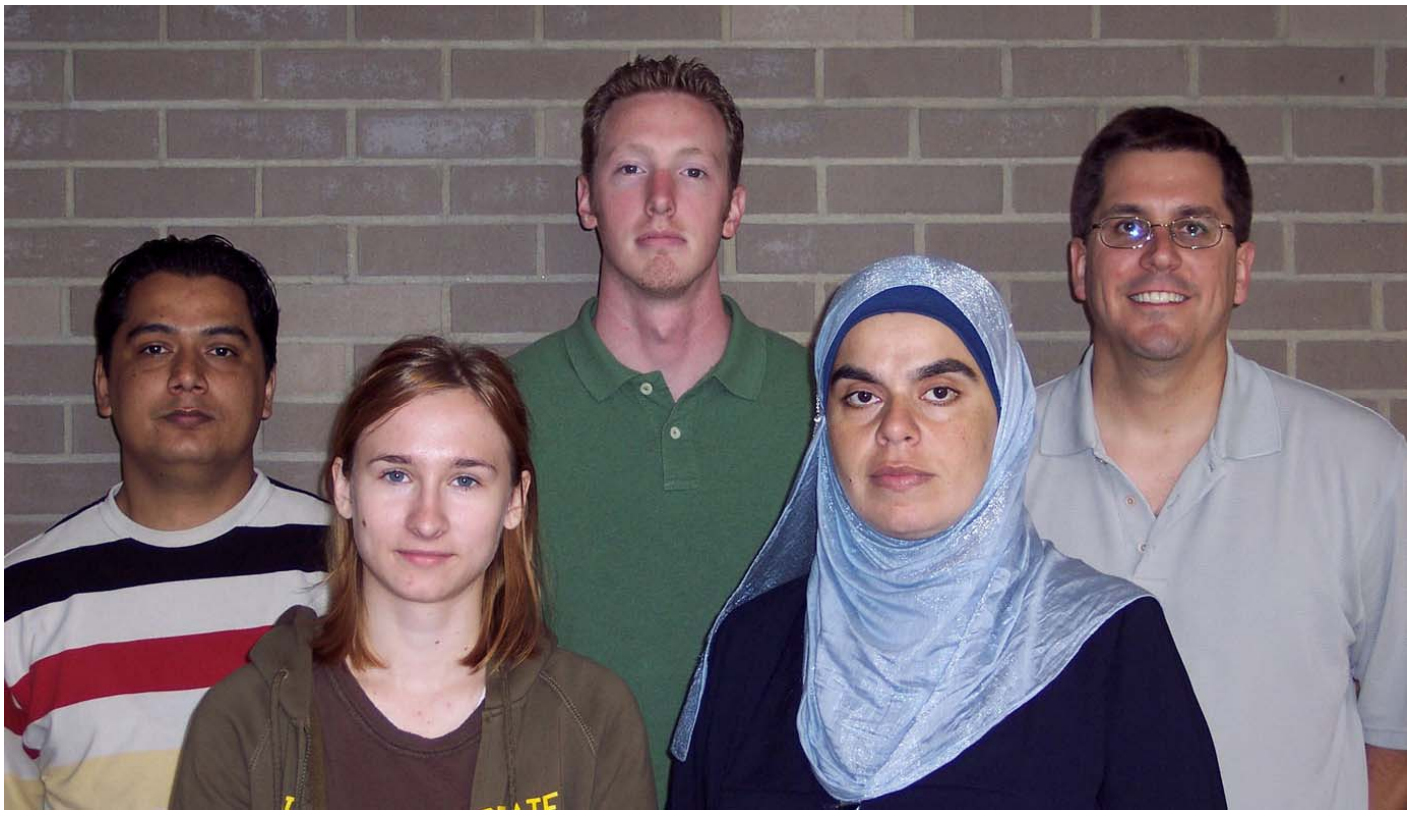
Conclusions

- 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.

- Early results show LIBS can be combined with Raman for improved accuracy of identification: “sensor fusion.”

My students



My funding…?

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