

Laser-Induced Breakdown Spectroscopy: A Novel Technology for the Rapid Detection, Identification, and Discrimination of Biological Agents

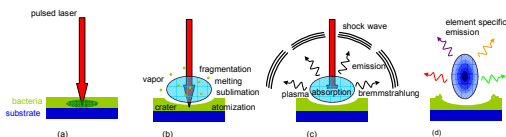
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Introduction

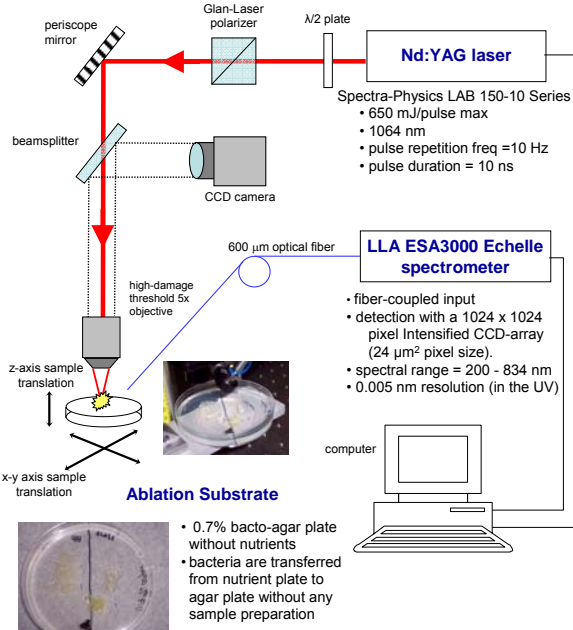
In the past several years **Laser-Induced Breakdown Spectroscopy (LIBS)** has been suggested as a rapid, field-portable technology for the identification of biological agents, particularly those agents related to bio-terrorism [1], [2], [3], [4], [5]. During LIBS an intense pulse of laser light vaporizes the target bacteria and spontaneous emission from the bacteria's constituent atoms is detected.



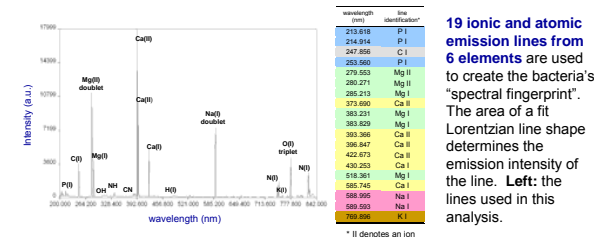
The analysis is based completely on the relative concentrations of trace **inorganic elements** located, in part, in the membrane of the bacteria. [6] It is NOT based on a genetic / DNA difference.

In the present work, LIBS was utilized to discriminate four strains of *Escherichia coli* and one strain of *Pseudomonas aeruginosa* cultured on three different growth media. [7] This is the first ever demonstration of **discrimination between strains** utilizing LIBS and the first investigation of the relationship that the growth substrate has on the LIBS analysis.

LIBS Elemental Microscope



E. coli LIBS Emission Spectrum



19 ionic and atomic emission lines from 6 elements are used to create the bacteria's "spectral fingerprint". The area of a fit Lorentzian line shape determines the emission intensity of the line. Left: the lines used in this analysis.

Bacteria / Nutrient Media

E. coli (4 strains, 2 media)

1. HF4714 (K-12)
2. AB (K-12)
3. Nino C (environmental strain)
4. enterohemorrhagic *E. coli* (EHEC)

trypticase soy agar (TSA) plate

24 hours, 37°

trypticase soy broth (TSB) vial

P. aeruginosa (1 strain, 3 media)

trypticase soy agar (TSA) plate

24 hours, 37°

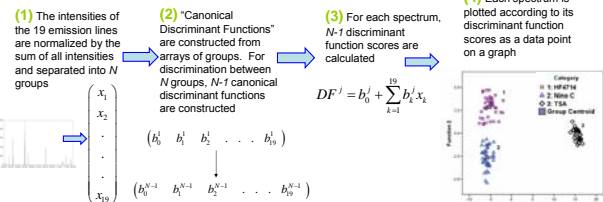
MacConkey agar (MAC + lactose) plate plus bile salts

blood agar (TSA + blood) plate

TSA + 0.4% bile salts (Sigma)

Discriminant Function Analysis

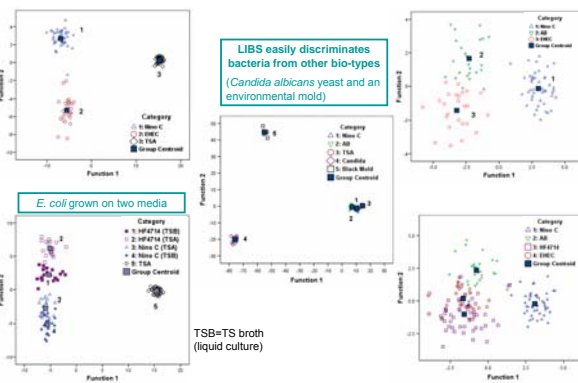
DFA is a mathematical / linear-algebra-based statistical analysis technique that expresses groups of data in terms of functions which maximize the difference between those groups.



[1] S. Morel, M. Leone, P. Adam, and J. Amouroux, "Detection of bacteria by time-resolved laser-induced breakdown spectroscopy," *Appl. Opt.* **42**, 6184-6191 (2003).
 [2] A.C. Samuels, F.C. DeLuca, Jr., K.L. McNesby, and A.W. Mziolik, "Laser-induced breakdown spectroscopy of bacterial spores, molds, pollen, and protein: initial studies of discrimination potential," *Appl. Opt.* **42**, 6205-6209 (2003).
 [3] F.C. DeLuca, Jr., A.C. Samuels, R.S. Hamon, R.A. Walter, K.L. McNesby, A. LaPointe, R.J. Winkler, Jr., and A.W. Mziolik, "Laser-induced breakdown spectroscopy (LIBS): a promising versatile chemical sensor technology for hazardous material detection," *IEEE Sens. Jour.* **50**, 681-689 (2005).
 [4] D.C.S. Beddows and H.H. Telle, "Prospects of real-time single-particle biological aerosol analysis: A comparison between laser-induced breakdown spectroscopy and aerosol time-of-flight mass spectrometry," *Spectrochim. Acta B* **60**, 1040-1059 (2005).
 [5] Jonathan Diedrich, Steven J. Rehse, and Sunil Palchadhuri, "Escherichia coli identification and strain discrimination using nanosecond laser-induced breakdown spectroscopy," accepted for publication, *Applied Physics Letters*, March 2007.
 [6] P. Singleton, *Bacteria in Biology, Biotechnology and Medicine*, 4th ed. (Wiley, Chichester, England, 1997).

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E. coli Strain Discrimination



P. aeruginosa Discrimination and Growth Medium Effect

