# Recent advances in the use of laser-induced breakdown spectroscopy (LIBS) as a rapid point-of-care pathogen diagnostic

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## ABSTRACT

Laser-induced breakdown spectroscopy (LIBS) has made tremendous progress in becoming a viable technology for rapid bacterial pathogen detection and identification. The significant advantages of LIBS include speed (< 1 sec analysis), portability, robustness, lack of consumables, little to no need for sample preparation, lack of genetic amplification, and the ability to identify all bacterial pathogens without bias (including spore-forms and viable but non-culturable specimens). In this manuscript, we present the latest advances achieved in LIBS-based bacterial sensing including the ability to uniquely identify species from more than five bacterial genera with high-sensitivity and specificity. Bacterial identifications are completely unaffected by environment, nutrition media, or state of growth and accurate diagnoses can be made on autoclaved or UV-irradiated specimens. Efficient discrimination of bacteria at the strain level has been demonstrated. A rapid urinary tract infection diagnosis has been simulated with no sample preparation and a one second diagnosis of a pathogen surrogate has been demonstrated using advanced chemometric analysis with a simple "stop-light" user interface. Stand-off bacterial identification at a 20-m distance has been demonstrated on a field-portable instrument. This technology could be implemented in doctors' offices, clinics, or hospital laboratories for point-of-care medical specimen analysis; mounted on military medical robotic platforms for in-the-field diagnostics; or used in stand-off configuration for remote sensing and detection.

**Keywords:** laser-induced breakdown spectroscopy (LIBS), point-of-care medical diagnostics, rapid pathogen identification, infectious diseases, chemometric analysis, stand-off detection

## 1. INTRODUCTION

Since 2003, considerable effort and resources have been devoted to developing the analytical technique of laser-induced breakdown spectroscopy (LIBS) for the rapid point-of-care diagnosis of bacterial and viral pathogen infection.[1,2] The numerous advantages of a LIBS identification (based on a part-per-million elemental assay), which include: protocols involving no consumables and little or no sample preparation, testing times of seconds, and the ability to analyze micrograms to nanograms of a specimen in solid, liquid (or "wet"), aerosolized, or powdered forms, have been described in numerous publications and papers.[3-5] In addition, the recent addition of computerized signal-processing techniques ("chemometric algorithms") for the autonomous classification and group-membership prediction of LIBS spectra has added the ability to make rapid threat-assessment/diagnoses on the basis of user-defined criteria.[6,7] The results of such predictions can be simply and unambiguously conveyed to non-expert first-responders or health–care providers in near real-time.[8]

While early investigations of the efficacy of LIBS for bacterial identification utilized very concentrated pure lyophilized bacterial samples and focused mainly on proof-of-concept demonstrations such as discrimination amongst biotypes,[9] identification of single cells/spores in bioaerosals,[10-12] and the use of molecular emission in the LIBS spectrum,[13] more recent investigations conducted in the last four years have attempted to investigate the utility of the technique for identifying live pathogens of high medical interest in "real-world" samples/specimens. In these experiments, careful consideration has been given to the microbiology involved (state of growth, nutritional medium, and chemical environment) as well as variations amongst the various taxonomic classifications of the bacteria (at the species or strain level). This article attempts to summarize the finding of those experiments to convey the robustness and accuracy of a LIBS-based bacterial identification.

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## 2. METHODOLOGY

### 2.1 LIBS apparatus

The majority of experiments summarized in this paper utilize an experimental procedure and apparatus very similar to that utilized in our laboratories. These are shown schematically in Fig. 1.

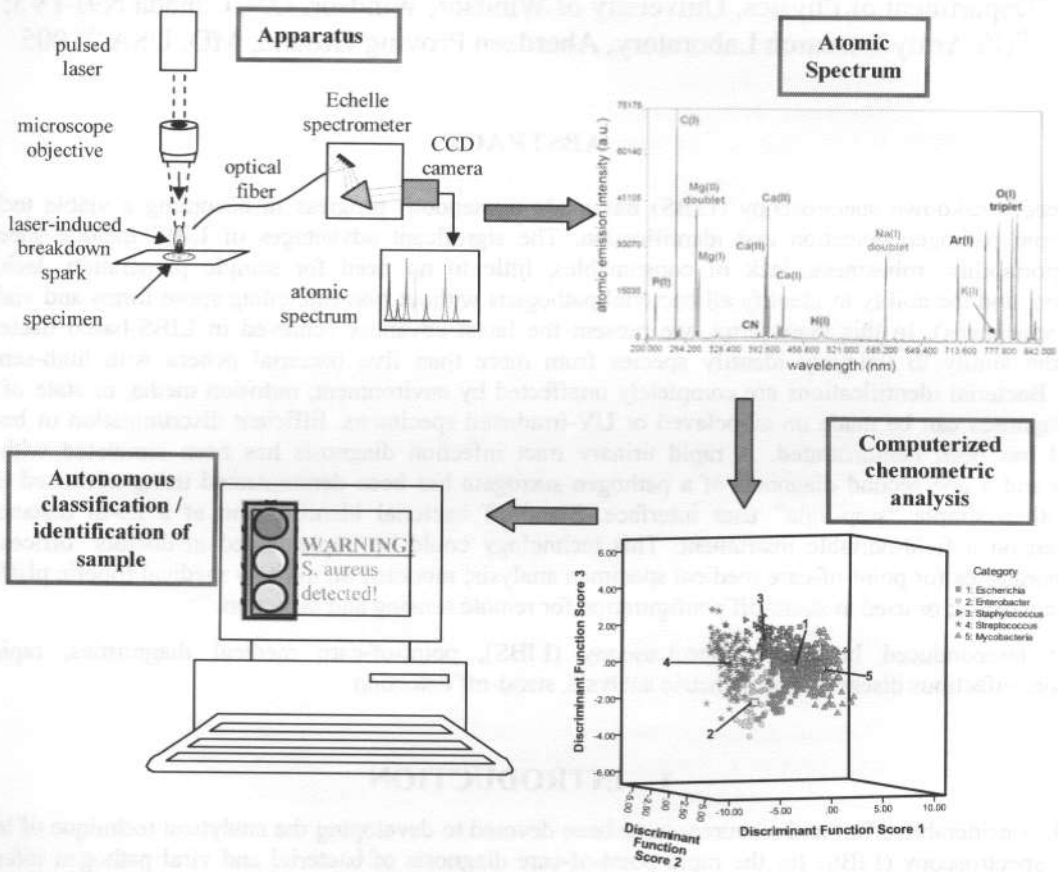


Figure 1. A schematic of how a laser-induced breakdown spectroscopy apparatus would be used for bacteriological applications. (top left) A bacteria-containing specimen is atomized by a focused (100 micron diameter) high-energy pulsed laser. Nd:YAG lasers are typical. Light emitted from a high-temperature spark is collected and dispersed. The atoms present in the specimen are all identified by peaks in the atomic emission spectrum (top right), which in this case was obtained from approximately 7500 bacterial cells. The ratios of the intensities of these peaks form a "spectral fingerprint" unique to the bacterium, which can act like a bar-code to identify the bacterium in the specimen. Advanced computerized classification algorithms analyze the LIBS spectrum, and based on the specimen's unique spectral characteristics, assign the unknown specimen to a class according to a precompiled reference library (bottom right). No user input is required, although complementary diagnostic information may be included to assist in spectrum interpretation/classification. The results of the diagnostic analysis are conveyed in real-time to the operator (physician, technician, etc.) unambiguously (bottom left). Because the fingerprint is obtained easily and quickly, in under one second, it can also be used to quantify changes in the specimen with time.

### 2.2 Bacterial composition

As noted, a bacterial LIBS identification is based on a sensitive elemental assay of the bacterial cell – often times discriminating between bacteria on the basis of trace inorganic elements and metals in the cell.[14] While the composition of a bacterial cell varies from species to species, the approximate or average composition is known. This is shown in Table 1. Also shown in column four of Table 1 is the percentage of the LIBS total spectral power (which is the total integrated area under the emission curve in the LIBS spectrum) attributed to that element as measured in a typical

bacterial specimen. It is important to note that the fraction of total spectral power observed in the LIBS spectrum is not a measurement of the total elemental concentration in the bacterial cell, because the relative emission strength of a given elemental emission line depends not only on the concentration of that analyte in the cell, but also on the relative transition oscillator strength of the atomic/ionic transition. Therefore the observed emission lines in the LIBS spectrum give a qualitative knowledge of the elements present (and changes in the peak intensities can be related to changes in the concentrations of those elements) but they are not currently used to quantify absolute concentrations in the cell. Also, the relative intensities observed are highly dependent on the atmospheric environment in which the LIBS plasma was created. The data shown are from spectra acquired in argon, which has been shown in numerous studies to provide an increased signal-to-noise ratio in the LIBS spectrum. The LIBS data are averaged relative emission intensities from 669 bacterial spectra acquired from bacteria with 13 different taxonomic classifications.[15] Approximately 32 different specimens (different cultures, tested over many months) are represented.

Table 1. Relative fractions of inorganic elements measured in bacterial cells and their contribution to the LIBS spectrum.

| Element | % of fixed salt fraction | % dry wt. basis | % of observed emission intensity in LIBS spectrum (see text) |
|---|---|---|---|
| | Gusnsalus and Stanier[16] | Aiba et al.[17] | This work |
| Carbon | NO | 50 | 35.0 |
| Sodium | 2.6 | 0.5-1.0 | 3.6 |
| Potassium | 12.9 | 1.0-4.5 | NO* |
| Calcium | 9.1 | 0.01-1.1 | 19.5 |
| Magnesium | 5.9 | 0.1-0.5 | 32.2 |
| Phosphorus | 45.8 | 2.0-3.0 | 9.8 |
| Sulfur | 1.8 | 0.2-1.0 | NO |
| Iron | 3.4 | 0.02-0.2 | NO* |
| Manganese | NO | 0.001-0.01 | NO |
| Copper | NO | 0.01-0.02 | NO |
| Zinc | NO | NO | NO* |

NO: not observed or not reported
NO*: not observed by Rehse et al.[15] but element has been observed by others in bacterial LIBS spectra

## 3. SUMMARY OF RESULTS

A condensed tabulated summary of results as reported in peer-reviewed literature in the last five years is presented in Table 2 and discussed below.

Table 2. Summary of recent experimental advances in LIBS-based bacterial diagnosis.

| |
|---|
| *(2007-2012)* The bacterial LIBS spectrum for a given species is stable and does not change with time (experiments conducted on the same *E. coli* strain over the course of multiple years). |
| *(2007)* A rapid discrimination of live bacteria on the basis of LIBS signature alone is possible, as well as discrimination from other biotypes such as yeast or mold.[18] |
| *(2007)* Discrimination of the pathogenic enterohemmorhagic *E. coli* O157:H7 strain from other |

non-pathogenic *E. coli* strains has been shown.[19]

*(2007&2011)* Bacterial identification appears to be independent of the growth condition and culture medium in which the bacteria were grown (a nutrient rich tryptic soy agar, broth, or blood agar medium).[20] This result has been confirmed by Marcos-Martinez et al. on three similar growth media *(2011)*.[21]

*(2008)* Detection and discrimination of the biological warfare agent anthrax surrogate *Bacillus subtilis* var. niger and ricin surrogate ovalbumin has been demonstrated with 0% false negatives and 1% false positives at 20 meters using a standoff system.[22]

*(2009)* Bacteria can be discriminated in air, argon, or helium environments, although argon and helium offer distinct advantages.[23]

*(2009)* Bacterial LIBS signatures are correlated with bacterial membrane composition (for Gram-negative bacteria).[14]

*(2010)* Discrimination is possible between three clonal methicillin-resistant *Staphylococcus aureus* (MRSA) strains and one unrelated MRSA strain.[24]

*(2010)* Intensity of the LIBS spectrum is linearly dependent on cell number, but the specificity is not dependent on cell number. 1500 cells provides adequate signal-to-noise.[25]

*(2011)* Bacterial LIBS spectra do not change with time as the bacterial culture ages on an abiotic surface (necessary for accurate identification/detection of surface contamination with swipes).[26]

*(2011)* Bacterial LIBS spectra can be obtained from killed (via autoclaving) or inactivated (via UV light) specimens, and such treatment (which renders the specimen completely safe for handling) does not decrease identification specificity and does not decrease LIBS spectral intensity.[26]

*(2011)* LIBS can differentiate and discriminate among bacteria in the un-mined and reclaimed chronosequence of bauxite soils, useful for the evaluation of soil quality and soil chemical alteration.[27]

*(2011)* Bacterial LIBS spectra can identify *Salmonella enterica* serovar Typhimuriumin at various concentrations in various liquids such as milk, chicken broth, and brain heart infusion. Titers of $10^5$ and $10^6$ cfu/mL provide adequate sensitivity for such testing.[28]

*(2011) Bacillus atrophaeus* spores and *Escherichia coli* are identifiable on a variety of testing backgrounds within a variety of residue mixtures of similar biological confusant material.[7]

*(2011&2012)* Bacteria in mixed samples are identifiable. The dominant or majority bacterial component of a two-component bacterial mixture is reliably identified provided it comprises 70% of the mixture or more. Trace mixture or contamination is insignificant.[25,15]

*(2012)* Bacteria can be identified with high sensitivity and specificity when specimens are obtained from clinical samples (e.g. sterile urine containing organic and inorganic solutes) without the need to remove other compounds present in the sample.[15]

*(2012)* Bacterial classification of a "spectral library" composed of spectra from five bacterial genera and 13 distinct taxonomic groups showed sensitivities of approximately 85% and specificities above 95% when classified in a five-genus model. Positive predictive values (PPV) of 95%, 60%, 92%, and 96% were shown for the genera: *Escherichia*, *Staphylococcus*, *Streptococcus*, and *Mycobacterium*.[15]

*(2012)* Live pathogenic *Bacillus anthracis* Sterne strain and *Francisella tularensis* can be differentiated regardless of mounting protocol (as lawn and/or colonies on agar, dilutions on agar, and dilutions on glass slides.)[29]

## 4. DISCUSSION

At present the experimental approaches utilized in obtaining the results described in Table 2 are varied, and no standard apparatus or sample preparation protocol has been described or is recommended. Different laser sources are still used throughout the community. Although the fundamental (1064 nm) of the nanosecond Nd:YAG laser is very common, other harmonics, as well as near-infrared Ti:sapphire femtosecond pulses are also utilized. Power fluences vary by experiment and by research group, different plasma emission collection geometries are used, different gas environments are present, and no standard protocol for bacterial specimen preparation yet exists. As such, there is still no standard agreement on what the "typical" LIBS spectrum is for a given organism (e.g. there is still no "standard" MRSA LIBS "spectral fingerprint" useable by labs world-wide.) This is also the situation through a large segment of the LIBS-community, although efforts to standardize assays are increasing. To date, no effort to standardize the identification of bacteria has been initiated. This situation is understandable given how recent these results are and given that efforts in multiple LIBS laboratories working on microbiological specimens are not yet well-coordinated. Still, there is recognition that LIBS research into microbiological identification and biological/biomedical analysis needs to move to the next step of standardization. This will occur as funding allows.

Quite possibly the most significant variation exists in the methods of data analysis. Even if common protocols were adapted, the nature of the chemometric algorithms used could greatly affect the technique's ultimate sensitivity and specificity. One of us (Rehse) has successfully implemented a discriminant function analysis (DFA) approach using the individual atomic/ionic emission lines observed in the LIBS spectrum as independent predictor variables, while the other research effort (ARL LIBS group) has had great success utilizing partial least squares-discriminant analysis (PLS-DA) approach utilizing not only the atomic emission lines, but also ratios of the observed lines and molecular emission as well. Both techniques have shown success. The process of utilizing the intensities of known emission lines as predictor variables is known as variable "down selection." Many groups, however, utilize the entire LIBS spectrum (typically on the order of 200-900 nm) with every spectrometer channel serving as an independent predictor variable. At present, there is still no standard of analysis. It has been observed by Gottfried et al. that use of the entire LIBS spectrum can be computationally intensive on spectra with many channels and this may also provide a discrimination based (incorrectly) on matrix effects, atmospheric lines, or background. Conversely, use of the down-selected emission intensities and ratio combinations can reduce the effects of shot-to-shot variability while allowing the user to construct models containing the most useful ratios and combinations.[7] We are at present testing the performance of DFA vs. PLS-DA using our 5-genus and 13 classification bacterial library[15] as well as comparing the predictive ability of utilizing only emission peak intensities versus utilizing combinations of ratios of those peaks in both chemometric techniques. Currently, the size of our LIBS spectrum (>50,000 channels) precludes its use without variable down-selection. The use of other chemometric algorithms, including principal component analysis (PCA)[27] and neural network (NN)[21] analysis is also still reported in the literature.

### 4.1 Hardware development

The ARL researchers have considerably advanced the standoff LIBS technology by closely working with a LIBS company (Applied Photonics, Ltd., Skipton, North Yorkshire, UK) which has designed and built five generations of standoff LIBS devices.[31] One of the many possible applications of a standoff system relevant to bacteriological identification would be the analysis of apparent casualties from a short distance, e.g. 1-3 meters, using a LIBS system mounted on a robot. This application, which can be broadly classified as "telemedicine," could interrogate open wounds and other bodily fluids that are in the line-of-sight of the LIBS system.

Important to hospitals, clinics, and emergency rooms, ARL researchers in conjunction with a signal processing company (New Folder Consulting, Durham, NC, USA) have developed and demonstrated advanced signal-processing software for the real-time (one second) LIBS analysis and PLS-DA classification of anthrax surrogates.[32] This benchtop system is controlled by a common laptop computer and conveys results to the operator in a simple, intuitive way. Although developed primarily for rapid CBRNE threat analysis, this hardware/software package could easily be adapted for clinical medical applications for point-of-care pathogen diagnosis.

## 5. CONCLUSION

Although currently a purpose-built commercial instrument for the real-time LIBS-analysis of biomedical/medical specimens does not exist, it is clear from the experiments summarized here that the authors and others have shown that at present the hardware and software exist to make such an instrument possible. As well, the necessary microbiological experiments have been conducted to show that a LIBS-based analysis is not only sensitive and specific, but is robust against many sources of error and uncertainty common to other methods: sample contamination, mixed samples, low cell count, etc. What is now required is the investment of resources to develop a purpose-built commercial instrument, the development of protocols for standardized bacterial preparation and mounting, and experimentation leading to clinical trials of the instrument (analysis of bacteria in or obtained from clinical specimens), as well as its protocols and chemometric classification algorithms in a clinical microbiology laboratory. These results can then be compared to the "culture and count" gold standard method.

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