

focus on Microscopy & Microtechnology

The potential for a rapid, field-deployable, automated biosensor detection system for pathogens

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Hospital acquired infections (HAI), often referred to as 'superbugs', are never far from the news. MRSA (methicillin-resistant *Staphylococcus aureus*) leads the way in press coverage but is just one of a growing group of bacteria that can cause diseases with increasingly limited therapeutic options. Organisms with resistant strains include vancomycin resistant *Staphylococcus haemolyticus*, *Clostridium difficile* (*C. diff*), and numerous enterococcal isolates with plasmid-mediated gentamicin resistance and vancomycin resistance. At a time of rising levels of MRSA and other hospital-acquired infections, rapid and reliable detection would offer an important safeguard to patients and staff alike.

Laser-Induced Breakdown Spectroscopy

Laser-induced breakdown spectroscopy (LIBS) is a spectroscopic analysis technique in which a focused laser pulse is directed onto a target. Whether a solid, liquid, or gas, the energy from the pulse vaporises, atomises and ionises the target material to form a micro-plasma, which emits light as a result of relaxation of electrons from excited to lower energy states. The spectral signature of the plasma holds the characteristic optical fingerprint of the individual elements within the target (Figure 1).

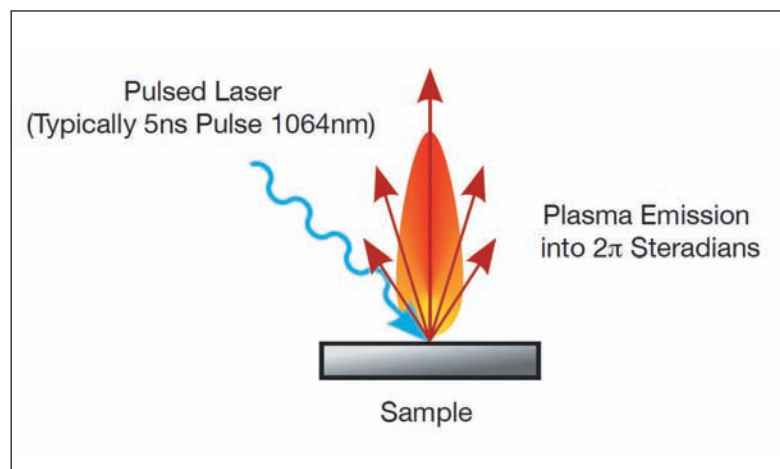


Figure 1. LIBS plasma plume generation

The laser pulse usually lasts for 5 to 20 ns and, typically, the emitted light is routed to an Echelle spectrograph, with its combination of high resolution and wide wavelength coverage. The spectrum observed in the first 100 ns is dominated by continuous, intense, white-light radiation that contributes to a high background signal, making the observation of discrete lines difficult. The plasma plume expands with time and the excited species relax further.

After a delay of approximately 1 μ s from the incident laser pulse, discrete spectral lines originating from various ionic species become increasingly visible as the high background emissions decay. The exact timing and the spectral lines vary with the type of sample, the distance from the centre of the plasma and the wavelength of the incident laser light. Typically, the evolution of the plasma and the changes in its content occur on a microsecond timescale and a precision, ultrafast camera is required – usually an intensified CCD (ICCD) system.

If the laser selected exhibits a good Gaussian profile, which allows focusing to a near diffraction-limited spot, LIBS does not require a large amount of energy. Typically, energies of only a few tens of mJ are necessary. In many ways, the analysis is similar to that performed by an ICP (Inductively Coupled Plasma) analyser but without the sample preparation that ICP requires.

It is the combination of minimal sample preparation and a low power requirement that makes LIBS a potentially valuable technique for automated use in the field and or unattended situations outside the controlled laboratory environment.

LIBS and Biological Samples

The LIBS group at Applied Research Associates is investigating the use of LIBS in a number of novel testing situations, including industrial process monitoring, environmental monitoring, and workplace surveillance for harmful materials, as well as deployment in space exploration.

In 2006, Matthieu Baudelet and colleagues [1] used processed LIBS spectra captured through a Mechelle Echelle spectrograph (Andor) to investigate the relative concentration of six trace elements in pure samples of five bacterial species and showed the equipment's suitability for accurate identification and discrimination.



Figure 2. The ARA team: (left to right): Rosalie A. Multari, David A. Cremers, and Melissa L. Bostian

The ARA group (Figure 2) used the iStar intensified CCD camera (Andor) and Echelle spectrograph to demonstrate for the first time that LIBS may be used to discriminate pure, viable pathogen samples based only on raw (unprocessed) LIBS spectra. This work was carried out in a collaborative research effort with a group led by Dr. John Gustafson of the New Mexico State University Biology Department (Figure 3). This collaborative work is the first blind study in which LIBS data was used to successfully identify five pathogenic bacterial samples and differentiate between strains of a multiple-antibiotic-resistant species [2]. The LIBS apparatus used to collect the data as set-up in the ARA laboratory is shown below (Figure 4).

Sub 60-second identification of MRSA and other HAIs

Multiple-antimicrobial-resistant methicillin-resistant *Staphylococcus aureus* (MRSA) causes serious infections in hospital patients and within the general populace and strains exhibiting reduced susceptibility to the principal anti-staphylococcal drug, Vancomycin, have been reported. Numerous time-consuming culture-media-based and molecular biology techniques are usually required to differentiate common bacterial pathogens, resolve their clonal relationship among single specie strain collections, and determine antimicrobial resistance profiles. Determining antimicrobial resistance phenotype is imperative when determining which antimicrobial regimen will best suit a diseased individual.

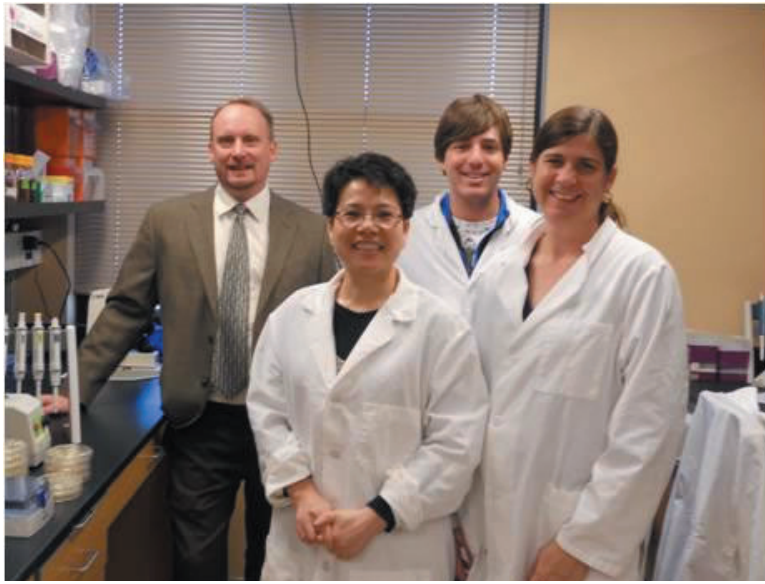


Figure 3. The NMSU team: (left to right in front) Wanqin Yu, JoAnne M. Dupre, (behind) John E. Gustafson and Jesus A. Cuaron

E. coli strain DH5cx and four strains of *Staphylococcus aureus* (LP9, MM61, MM66, and MM66-4) were chosen for the study. All the bacterial cell samples were grown, harvested, and lyophilised by researchers at New Mexico State University. In total, 15 unidentified samples in disposable cuvettes were presented to the ARA team and they were told that the first five samples were unique and the remaining ten samples were replicates, two each, of the first five. LIBS spectra were collected by focusing pulses from a Q-switched Nd:YAG laser (1064 nm, 60 mJ/pulse, 10 Hz) into the open end of a cuvette contained within a Class II biological safety cabinet and sparking the pathogenic sample within.

Each spectra was the accumulation of ten spectra from the laser-induced plasma plumes, with the collection of each spectrum accurately delayed by 1 microsecond from the laser pulse and integrated on a 20 microsecond temporal scale. Because the sample identities were unknown and to mimic an analysis situation in which data are collected and not controlled for quality, all collected spectra were used in the analysis with no screening for spectral quality and no data preprocessing. A total of 1050 accumulated spectra (70 spectra datasets for each sample) over the entire spectral range of 205.42 to 1000 nm from individual samples were collected. The LIBS classification spectra from Sample B (*E. coli*) is shown in Figure 5. The units of the ordinate axis is detector counts.

By using chemometric analysis of the LIBS spectra in combination with an identification algorithm, the ten blind samples were correctly matched to the unique samples thus demonstrating the spectral identification of all five bacterial samples with 100% accuracy.

The iStar Intensified CCD camera is perfectly adapted to cope with these challenging LIBS measurements, equipped with a fully integrated, software-controlled digital delay and ultrafast and ultralow jitter electronics for sub-2ns to ms optical shuttering capabilities. Coupled with the Echelle spectrograph, the camera delivers the highest spectral and time resolution across a very large bandwidth.

A new generation of rapid, automated biosensor detection systems?

This study demonstrates that LIBS can be used to differentiate the common hospital-borne bacterial pathogens, *E. coli* and *S. aureus* in pure form in less than a minute with 100% accuracy using only the raw LIBS spectra in an automated system. Furthermore, in combination with appropriately constructed chemometric models and defined testing flows, LIBS could be used to successfully classify an unknown pathogen in pure form, both species and strain, provided the unknown pathogen is within a defined set of pathogens. In medical treatment applications, by classifying pathogens in matrices of interest (such as blood or tissue), this capability could possibly be used to create testing algorithms to assist in rapid pathogen identification, thereby speeding the initiation of an appropriate antimicrobial-therapeutic regimen.

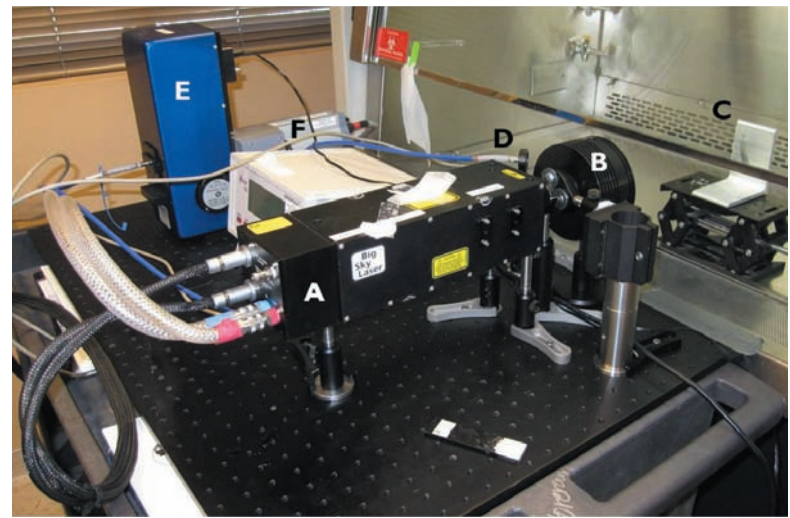


Figure 4. LIBS setup at ARA: (A) Nd:YAG laser, (B) laser focusing optics, (C) biological target in Class II safety cabinet, (D) collection fibre optics, (E) Echelle spectrometer, (F) Andor iStar ICCD

As a 'proof-of-principle' for a LIBS-based instrument for rapid pathogen detection, these are important results since LIBS has many advantages as a biosensing method which include: little or no sample preparation; simplicity of use (focus the laser pulse on the material and collect the light); and rapid in situ analysis with results in less than a minute with automated analysis.

The potential for saving lives through the development of rapid diagnostic instrumentation that can be operated by personnel without any specific technical or LIBS expertise should not be underestimated.

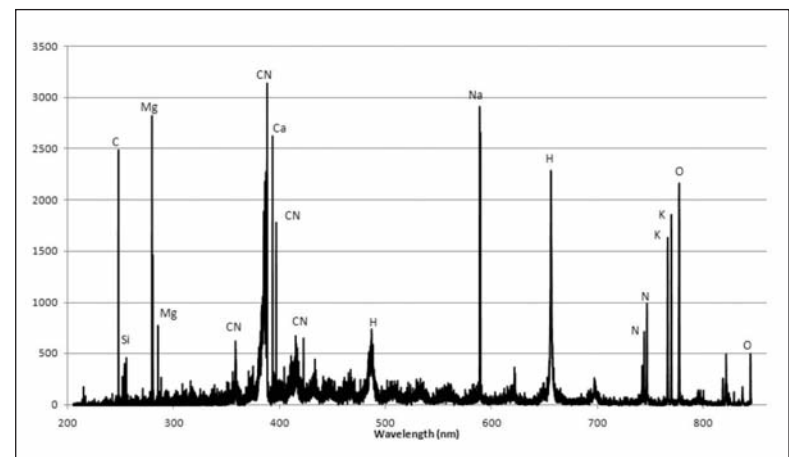


Figure 5. LIBS classification spectra from *E. coli*

References

1. Baudelet, M, Yu, J, Bossu, M, Jovelet, J. and Wolf, J. P. 'Discrimination of Microbiological Samples using Femtosecond Laser-Induced Breakdown Spectroscopy', *Applied Physics Letters* 89, 163903 (2006)
2. Multari, R. A, Cremers, D.A, Dupre, J. M. and Gustafson, J. E. 'The Use of Laser-Induced Breakdown Spectroscopy for Distinguishing Between Bacterial Pathogen Species and Strains', *Applied Spectroscopy* 64 (7), 750-759 (2010)

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Camera Technology Debuts at Daytona 500

FLIR Advanced Thermal Solutions has developed a state-of-the-art IR camera system called the 'Heat Seeker' that has enabled broadcaster - Fox Sports to record the extreme temperatures which develop on NASCAR motor vehicles during their recent Daytona 500 live broadcast. The thermal images are of particular interest as cars running in the rear of a piggy-backed pair lose air flow while drafting and must eventually switch places with the lead car to avoid overheating their engine. The technology can also reveal the on-track groove cars are using, as well as the difference between tyres about to be changed compared to fresh replacements. FLIR cameras were featured in three live segments during the Daytona 500 race: one segment that compared a hot tyre just taken of a car to a cold tyre sitting in the pits, the second segment compared the hoods of two cars during the race, and the final segment showed a blown out tyre just after being removed from a car late in the race.

FLIR supplied two of its SC8300 HD thermal cameras to Fox Sports to enable it to cover the race. One camera was configured to be portable and used by a roving Fox camera man in the pits. The other camera was mounted on a tripod at Turn 4 approximately 100 feet from the track. The pit camera was used to acquire thermal images of the activity in the pits and the Turn 4 camera was used to take video of the cars as they passed by at over 200mph. Every day researchers throughout the world are looking for new methods to help them solving their scientific questions. Thermal imaging using IR cameras' has proven to be an invaluable tool to solve a wide variety of scientific problems based on heat and temperature measurement. Because of its non-intrusive analysis capacity, thermal imaging systems have become indispensable instruments for a wide variety of research and development applications.

