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REVIEW

Laser-induced breakdown spectroscopy (LIBS): an overview of recent progress and future potential for biomedical applications

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The recent progress made in developing laser-induced breakdown spectroscopy (LIBS) has transformed LIBS from an elemental analysis technique to one that can be applied for the reagentless analysis of molecularly complex biological materials or clinical specimens. Rapid advances in the LIBS technology have spawned a growing number of recently published articles in peer-reviewed journals which have consistently demonstrated the capability of LIBS to rapidly detect, biochemically characterize and analyse, and/or accurately identify various biological, biomedical or clinical samples. These analyses are inherently real-time, require no sample preparation, and offer high sensitivity and specificity. This overview of the biomedical applications of LIBS is meant to summarize the research that has been performed to date, as well as to suggest to health care providers several possible specific future applications which, if successfully implemented, would be significantly beneficial to humankind.

Keywords: Laser-induced breakdown spectroscopy, Medical diagnostics, Point-of-care, Rapid pathogen identification, Infectious diseases

1. Introduction

Laser-induced breakdown spectroscopy (LIBS) has gained a reputation as a flexible and convenient technique for rapidly determining the elemental composition of samples with minimal or no sample preparation. Several excellent books have been published recently describing the latest advances in the field of LIBS [1,2,3]. The ability to easily determine the concentrations of trace analyte elements down to the part-per-million level (sub-ppm concentrations are also measurable by complementing LIBS with other techniques) has allowed LIBS to be utilized for assays of solids, liquids and gases in a wide variety of applications [4,5]. A sensitivity to all the elements without bias (e.g. to the lighter elements) and the use of powerful computerized chemometric techniques have allowed LIBS to be used for the rapid identification and/or discrimination of unknown materials. This has proven to be particularly useful when the unknown material needs to be rapidly classified as a threat (explosive, chemical or biological) or a non-threat. LIBS becomes even more attractive when this classification needs to be performed with a man-portable device [6,7], remotely (via a robotic or remote-controlled platform), or at stand-off distances (where the target and laser source are physically separated by distances up to 100 m [8]).

In the last few years, LIBS has begun to be applied more extensively in biological systems. For example, a wide variety of woods, trees, plants and vegetal tissues have been analysed to measure high-resolution variations in cellular biochemistry and the accumulation of trace metals *in situ* [9,10,11,12,13,14,15]. All of these applications make use of the ability of LIBS to rapidly quantify the organic and inorganic cellular composition of a target with high (~1–100 μm) spatial resolution in a rapid, reagentless analysis. A recent review describes the theory of the LIBS technique in detail and also compares the use of LIBS to other spectroscopic techniques for several of these applications [16]. A schematic of how a LIBS-based specimen elemental analysis or diagnostic identification is performed is shown in figure 1.

Also of interest has been the ability of LIBS to rapidly monitor the elemental composition of fruits and their aqueous extracts to determine their contribution to the known antidiabetic activities of these foods [17,18,19]. This information provided by LIBS concerning elemental concentrations has been correlated with a known efficacy for diabetes

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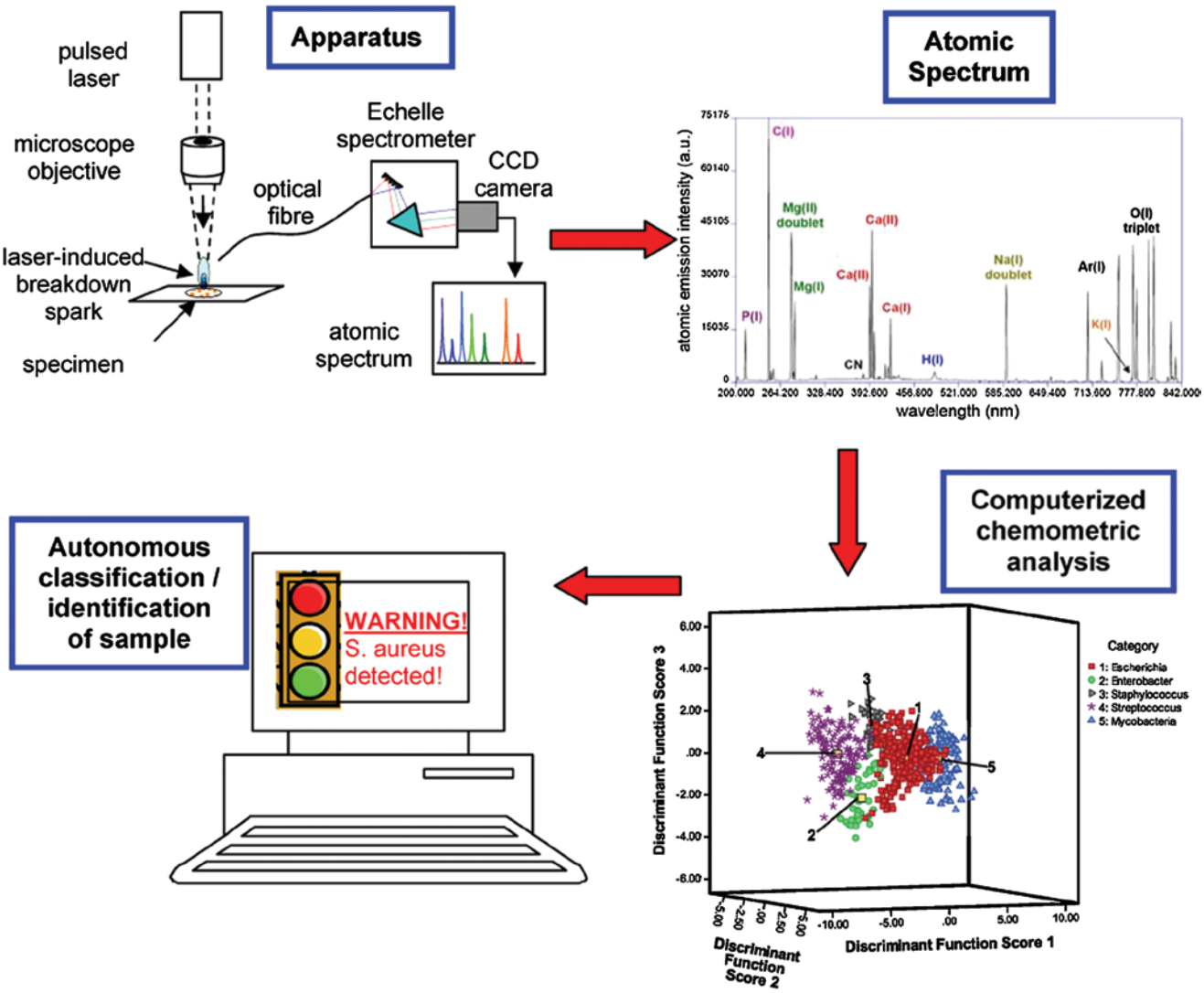

Figure 1. A schematic of how a laser-induced breakdown spectroscopy apparatus would be used for biomedical applications. (*top left*) A biomedical specimen (which can be presented in many forms, i.e. bacterial culture, tissue biopsy, bodily fluid specimen, disposable swipe, etc) is atomized by a focused (approximately 100 μm diameter) high-energy pulsed laser. 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 specimen, which can act like a bar-code to identify the specimen, or to quantify trace biomarkers in the fingerprint indicative of some unique pathology, disease state, or condition. (*bottom right*) Advanced computerized classification algorithms analyse the LIBS spectrum, and based on the specimen's unique spectral characteristics, assigns the unknown specimen to a class according to a precompiled reference library. No user input is required, although complementary diagnostic information may be included in the computerized model to assist in spectrum interpretation/classification. (*bottom left*) The results of the diagnostic analysis are conveyed in real-time to the operator (physician, technician, etc). 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.

management in both normal and diabetic animal models, extending the biomedical usefulness of the LIBS technique.

One of the rapidly expanding areas of research in bio-LIBS concerns the biomedical applications of LIBS: its use on human tissue samples or in systems relevant to human health and disease. The biomedical applications of LIBS can be broadly classified into two categories: (1) analysis of human clinical specimens (which may be teeth, bones, tissue samples, blood or other fluid samples) and (2) analysis of microorganisms (e.g. bacteria, moulds, yeasts) that can infect human subjects and cause disease. The use of LIBS to study hydrogen composition in proteins (which could be relevant to human health) has been suggested recently [20,21], but insufficient work has been done in this area to determine if this will be an emerging area for LIBS research.

All of these applications, as well as the general increased level of interest in LIBS-based biomedical analyses, are motivated by the inherent strengths of LIBS, which include the ability to perform an inherently real-time analysis that requires minimal or no sample preparation and no chemical reagents, biomolecular probes or markers, antibodies, or genetic amplification. Coupled with a demonstrated high sensitivity and specificity, a LIBS-based analysis offers the promise of a truly versatile real-time point-of-care diagnostic instrument for a wide variety of important applications.

2. LIBS on human tissues

2.1. Dental/mineralized tissues

Due to the ever-increasing and well-established use of lasers in clinical dental practice, LIBS was recognized very early as a potentially useful tool for the analysis of calcified tissues such as teeth, particularly the ability to discriminate caries tissue (cavities) from healthy tooth tissue but also to monitor the accumulation of trace metals in teeth [22,23]. LIBS can be performed *in vivo* or on removed and cross-sectioned tooth samples to create one-dimensional (depth) or two-dimensional maps to determine concentrations as a function of location or depth (associated with elemental penetration ability and diffusion time). This allows the study of the migration or distribution of elements within the calcified tooth matrix. It can also be used for the diagnosis of diseased or caries tissue in the otherwise healthy tooth. Specifically, it was an increased Mg concentration which clearly identified the caries tissue compared to the healthy tissue in a study by Samek *et al.* [24] An example of the LIBS spectra from this analysis is presented in figure 2. The LIBS results were later confirmed by atomic-absorption spectroscopy (AAS) measurements. This discrimination could allow a real-time and painless analysis of dental tissue during a drilling procedure.

The solid nature of hard mineralized tissues and their ability to be easily cross-sectioned make them particularly amenable to analysis by LIBS, compared to human soft-tissues which possess a higher water content. LIBS has been utilized to elementally characterize removed gallstones [25,26], kidney stones [27], and other urinary calculi (stones [28]). Rather than possessing a common composition, all of these stones occur in a wide variety of elemental concentrations and compositions, each uniquely identifiable via LIBS analysis. The identification of the elemental composition of the stone is a key step in the medical diagnosis of the condition which caused the formation of the stone. Thus LIBS could be an important tool for clinical laboratories. Such a spectro-chemical analysis of bone or other calcified tissue could also be relevant in the fields of forensic science and archaeology [29].

2.2. Soft tissues

Soft tissues possess a similar elemental composition no matter which organ they are obtained from. Yueh *et al.* [30] performed LIBS on tissue specimens from the brain, lung, spleen, liver, kidney and skeletal muscle of 20 normal six-week old chickens and determined that LIBS can discriminate between tissues and assist in the identification of the organ and tissue type. Unidentified 'test' spectra were then compared to positively identified reference spectra and classified using a variety of chemometric techniques commonly used with LIBS analysis, such as hierarchical cluster analysis (HCA), partial least squares discriminant analysis (PLS-DA), and neural network analysis (NNA). These tests proved that the tissue types were all differentiable from each other, but these authors concluded that identification of unknown samples required data with better reproducibility in order to produce reliable and robust

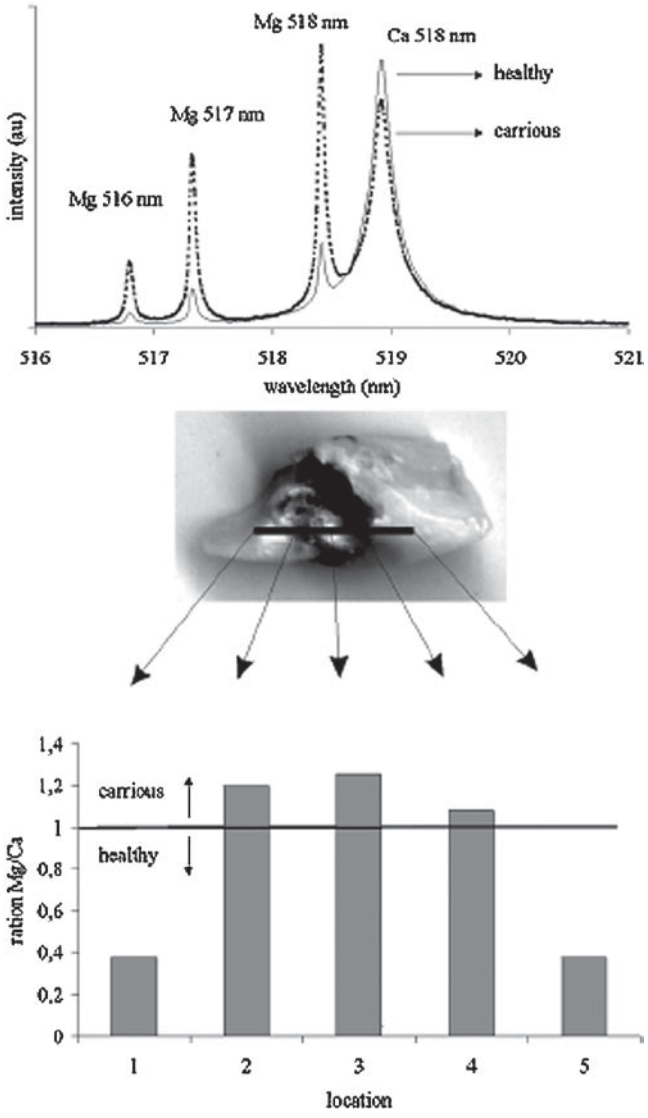

Figure 2. One-dimensional measurement map for the ratio of Mg content to Ca content, for a section of a caries-infected tooth from Samek *et al.* [24]. The increased Mg concentration clearly identifies the part of the tooth 'softened' by caries. (adapted from [24].)

classification models. The LIBS spectra acquired in this study, as well as the results of the NNA classification, are shown in figure 3.

This result is encouraging, but highlights the difficulties in performing sensitive elemental composition analysis on samples that exhibit a normal level of biodiversity. The intrinsic heterogeneity of tissue can lead to scatter in test data and validation data. Such difficulties will be encountered in any biomedical use of LIBS which attempts to classify or identify a biological sample. Due to this, caution must be taken when choosing the appropriate analyte emission lines in the LIBS spectrum (which can contribute to overfitting), when deciding how to treat outliers that occur in the validation data, when deciding how many model spectra are to be included in the validation datasets, and when choosing external validation set criteria.

Despite these challenges, LIBS has already been used to effectively distinguish normal and malignant tumour cells

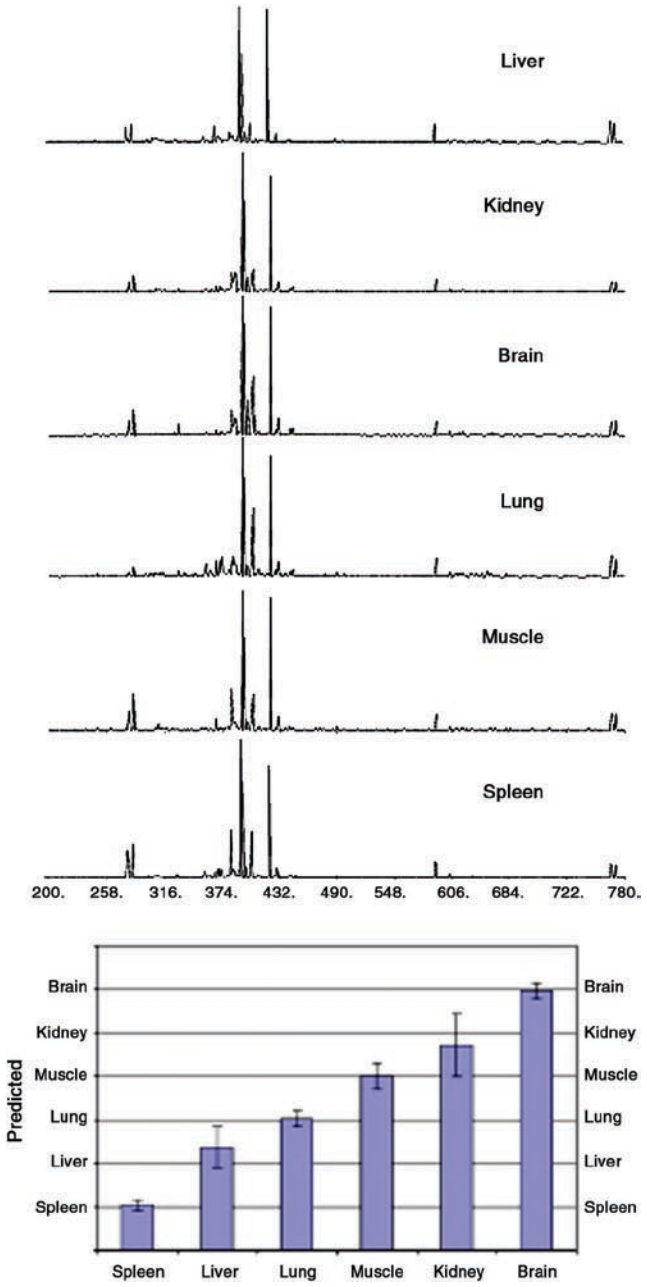

Figure 3. LIBS tissue spectra and the results of a neural network analysis classification from Yueh *et al.* [30]. (Adapted from reference [30].)

from histological sections [31] and to characterize several types of human malignancies [32], to determine the trace metal concentrations in human hair and skin samples [33,34,35], to determine the elemental composition of liver, kidney, muscle, and hepatopancreas tissues [36], and even to perform screening of whole human blood [37]. The usefulness of LIBS for characterizing or identifying human soft tissues for disease diagnosis is exemplified by the small amount of sample required and the potential ability to perform a LIBS analysis *in vivo* by delivering the excitation laser pulse and collecting the plasma emission through a single optical fibre probe [38]. This has already been demonstrated, even when the fibre and LIBS plasma are submerged underwater [39]. Therefore the ability to perform these analyses *in vivo* is theoretically possible. To date this type of analysis has not been performed. Still, this particular utilization would make the LIBS approach a recognizable technology to clinicians already familiar with laser-based endoscopic tools.

3. LIBS of microbes responsible for human disease

3.1. A global market

Perhaps the greatest amount of research into the biomedical application of LIBS has come in the area of identifying pathogens responsible for infectious human disease. A point of care diagnostic technology that can rapidly detect and identify pathogenic microorganisms that cause human disease is greatly needed by health care providers and many segments of medical society. There is currently an urgent need for an independent verification of bacterial identity that does not require *a priori* knowledge of nucleic acid sequences (required for polymerase chain reaction (PCR)-based techniques) or antibodies against known bacterial antigens (which fluorescence immuno-assay techniques require). To be maximally flexible, this technology should be able to identify pathogenic bacteria on surfaces and in clinical samples from patients at 'time zero' (the time when a clinical sample of blood, urine, cerebrospinal fluid, or sputum is obtained) with little or no sample preparation. The current research indicates that LIBS may be one of the candidate technologies to fill this role.

According to a 2007 study, three application areas account for over two thirds of all research in the field of (non-LIBS) pathogen detection: the food industry, water and environment quality control, and clinical diagnosis [40]. Military-biodefence constitutes a small niche market for this technology. Because of the global demand for pathogen detection technology and testing, as of 2003 the pathogen specific testing market was expected to grow for all segments at a compounded annual growth rate (CAGR) of 4.5% with a total market value of $563 million [41] ($US are used throughout this Review). In 2008, the annual worldwide investment in advanced biosensor R&D was estimated to be $300 million. The world biosensor market was $7.3 billion in 2003 and was expected to exceed over $10 billion by 2007 with the medical/health area being the largest sector [42].

3.2. LIBS progress: 2003–2005

The advantages of LIBS previously mentioned, particularly the speed of the diagnosis and the ability to make the instruments field portable (something not possible with a majority of competing technologies such as laser-ablation matrix-assisted laser-desorption ionization time-of-flight mass spectrometry (LA-MALDI-TOF) biotyping instrumentation) suggests its usefulness in this application. Making use of the advances in lasers, spectrometers, and applied chemometrics which were supported in large part by funding from the US Army, researchers from a half-dozen institutions in the time period 2003–2005 proved the feasibility of using LIBS to identify microorganisms including bacteria, yeasts, moulds, spores on surfaces, and airborne bioaerosals [7,43,44,45,46,47,48,49,50,51].

In all of these experiments, it was the measured inorganic elemental composition that provided the requisite uniqueness to reliably identify the microbe on the basis of its LIBS spectrum. Atomic emission from calcium, magnesium, phosphorus, sodium, potassium, carbon and iron as well as molecular emission from molecules such as CN, OH, and NH were used to create unique 'spectral fingerprints'. These spectral fingerprints were obtained from pure cultures of known specimens to create a target library, and then spectra obtained from unknown specimens were identified on the basis of a classification against the pre-compiled library. In this application of the technique, LIBS is most appropriately used to screen samples against a library of well-characterized commonly encountered or 'expected' organisms. However, the majority of the chemometric routines utilized in this application are capable of associating a previously unencountered organism with the organism or organisms most spectrally similar to it. Thus it is not necessary to create a library with every possible pathogen represented in it. For example, in our own studies, we have shown that different species of a given genus (e.g. *Streptococcus* and *Staphylococcus*) possess closely related LIBS spectra and were similarly identified in a discriminant function analysis (DFA [52]). This indicates that if a previously unfingerprinted species or strain (e.g. a *Streptococcus* species) was analysed, the DFA would most-likely identify it as belonging to the genus *Streptococcus*, not just return a 'pathogen unidentifiable' or null result. Importantly, the identification of a pathogen's genus is often enough information to begin treatment.

3.3. LIBS progress: 2005–2011

Since all the preliminary experiments indicated that it is indeed feasible to identity pathogens on the basis of their unique LIBS spectral fingerprint, follow-on experiments were conducted, and are ongoing, to translate the technology into a practical and fieldable diagnostic tool. Extensive work has been done to investigate the ability to identify pathogens present on surfaces [53], particularly to do this via 'stand-off' detection [8,54]. In particular, the military applications of bio-LIBS detection devices have been investigated extensively by the US Army Research Laboratory and others, as LIBS conveniently provides a 'platform technology' capable of identifying a broad spectrum of CBRNE (chemical, biological, radiological, nuclear, and explosive) threats. An important result of this work has been the demonstration of the ability to identify and discriminate bacterial pathogens or pathogen surrogates in the presence of complex interferents and substrates, even interferents of similar elemental composition [55,56].

The ARL researchers have considerably advanced the stand-off LIBS technology by closely working with a LIBS company which designed and built five generations of stand-off LIBS (ST-LIBS) devices [57]. One of the many possible applications of a stand-off system could be the analysis of apparent casualties from a short distance, e.g. 1–3 metres, using a LIBS system mounted on a robot which can interrogate open wounds and other bodily fluids that are in the line-of-sight of the LIBS system.

An intriguing variation on stand-off LIBS has been the use of femtosecond laser pulses, 'femto-LIBS', which can propagate without divergence through the atmosphere via spontaneous 'filamentation'. It has been shown that these filaments can be intense enough to produce breakdown plasmas and that biological agents can be identified in these LIBS plasmas [58,59]. This implies that stand-off detection and identification can be extended significantly further from the laser source than is possible with nanosecond laser pulses, and that complex focusing systems required to bring the laser to a focus on the sample are not required. To that end, the use of femtosecond laser pulses has been studied to determine its ability to discriminate microbiological targets [60,61], to compare its performance with nanosecond laser pulses [62], and to exploit the observation of molecular CN signatures in the femtosecond LIBS plasmas [63].

Recent work has also shown that LIBS-based identification of bacteria may be applied to non-clinical industrial applications as well. In the food-preparation industry, the utility of using LIBS to rapidly identify bacteria responsible for food-borne contamination such as *Salmonella enterica* serovar Typhimurium [64] has been demonstrated. In the mining or environmental remediation industry, it has been shown that femtosecond LIBS can be used to discriminate inter- and intra-site differences in soil bacteria (at both the species and strain level) isolated from mining sites as an indicator of environmental soil quality [65].

3.4. EMMA

Since 2006, our lab at the University of Windsor (formerly at Wayne State University) has been investigating the use of laser-induced breakdown spectroscopy to rapidly and accurately identify pathogenic bacteria specifically for clinical applications in hospital microbiology laboratories or military field hospitals. We call this LIBS-based identification of pathogens an 'Elemental Multivariate Microbiological Analysis', or EMMA. In these experiments, 10 ns 1064 nm laser pulses were used to ablate a small number of live bacteria in a sample that required no sample preparation or additional staining, specific monoclonal or mono-specific antibodies or DNA probes, and no primer or genetic amplification. Bacteria in 10 μl of a pure or mixed bacterial slurry were immobilized on the surface of an agar bed (assumed to form a uniform layer of bacteria). The infrared pulsed laser was focused to a spot size of approximately 100 μm diameter. The optical emission from the LIBS plasma (from 200 to 840 nm) was collected by an optical fibre located about 1 cm away, dispersed in an Échelle spectrometer coupled with an intensified-CCD detector, and recorded on a computer for analysis. Although initial experiments were performed in an air atmosphere, subsequent experiments were performed in an enclosed argon environment. Approximately 1500–7500 bacterial cells in total were required to make an accurate diagnosis (spread out under five separate laser sampling locations; 300–1500 cells per sampling location). LIBS spectra were classified via a discriminant function analysis utilizing the intensity (integrated area under the peak) of 13 atomic emission peaks that had been normalized by the total spectral power (defined as the total integrated area under the 13 measured peaks). This normalization reduced the influence of shot-to-shot laser energy variations or differences in

specimen titer (bacterial count) which can cause in spectral intensity variations.

We have already performed a number of initial experiments as reported in our first seven publications demonstrating the utility of this technique for clinical applications. We are vigorously investigating all of the microbiological variety that can possibly be induced in the bacterial cells in the expectation that this biodiversity will undoubtedly be encountered if EMMA is used clinically. A summary of the results we have demonstrated so far includes:

- A rapid discrimination of bacterial LIBS signatures from other biotypes such as yeast or mould is possible [66].
- Discrimination of *E. coli* strains, particularly the ability to discriminate the pathogenic enterohemmorhagic *E. coli* O157:H7 strain from other non-pathogenic strains, is possible [67]. LIBS-based strain differentiation was also demonstrated by Multari *et al*. [85] in *Staphylococcus aureus*, which is discussed in detail later in this Review.
- Our studies to date show that 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, or broth, or a blood agar medium [68]). This result has been confirmed by Marcos-Martinez *et al.* [69] on three similar growth media.
- Bacterial LIBS spectra do not change with time as the bacterial culture ages on an abiotic surface (necessary for accurate identification and detection of surface contamination [70]).
- Bacterial LIBS spectra can be easily 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 [70].
- Intensity of the LIBS spectrum is linearly dependent on cell number, but the specificity is not dependent on cell number [52].
- All species of bacteria tested to date have possessed unique atomic compositions allowing a LIBS-based identification of unknown bacterial specimens [52].
- 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 [52].
- Bacteria can be identified 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 (manuscript in preparation).
- Bacteria can be discriminated in air, argon, or helium environments, although argon and helium offer distinct advantages [71].
- Bacterial LIBS signatures are correlated with bacterial membrane composition (for Gram-negative bacteria [72]).
- 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).

Shown in figure 4 is a discriminant function analysis graph showing the first two discriminant function scores in a 2D plan-view and the first three discriminant function scores in a 3D view of over 600 bacterial LIBS spectra from 13 bacterial specimens representing five genera including *Escherichia*, *Enterobacter*, *Streptococcus*, *Staphylococcus* and *Mycobacterium*. In such figures, each data point represents the entire information content of a LIBS spectrum. By analysing the differences and similarities in LIBS spectra, the discriminant function analysis can rapidly and autonomously identify unique repeatable spectral characteristics in the LIBS spectrum, which then allow unambiguous identification of unknown spectra.

At no time was any relationship between the bacterial specimens input into the chemometric analysis shown in figure 4, yet it is obvious that spectra from similar bacterial specimens cluster closely together (i.e. *Staphylococcus* species are grouped together, *Streptococcus* species are grouped together, and *E. coli* strains are closely grouped together). This clustering is evidence of the consistency of the elemental diversity that exists between bacterial specimens, and also demonstrates the degree of sensitivity and specificity available in an EMMA discrimination. Taken in aggregate, all these results indicate that the use of a LIBS-based EMMA technology is feasible for the rapid detection and diagnostic identification of pathogenic bacteria with high sensitivity and specificity.

4. Applications of EMMA

4.1. Benchtop devices for clinical/quality assurance labs

Work is currently underway to design sample preparation protocols that will allow rapid pathogen detection/identification in almost any liquid sample. Blood, cerebral spinal fluid, urine, bronchioalveolar lavage, amniotic fluid, joint fluid, and vitreous fluid are the most important fluids that will need to be tested. Isolation of the bacteria in these samples is required by almost all pathogen biodetection technologies, including a LIBS-based EMMA technology. In the clinical lab, simple centrifugation and filtration protocols (taking only a few minutes) may suffice to concentrate bacteria from clinical specimens to allow their detection/identification by LIBS without the need for culture. After this simple preparation, the concentrated pathogen would then be placed in the EMMA benchtop unit for analysis. Research is ongoing to develop the EMMA 'front-end' technology that is needed to concentrate the bacteria present in a fluid sample (approximately 10 ml) and introduce these bacteria into the focused laser spot. The entire process from the time the fluid is obtained until pathogen detection/identification should take no more than 10 minutes, with the tests requiring only minimal technical skill. This rapid test would be extremely useful for the analysis of cerebral spinal fluid (CSF) for rapid meningitis diagnosis or for rapid urine screening for the diagnosis of urinary tract infections (UTI). Testing and treatment of UTIs is a broad area of clinical diagnosis where advances in pathogen detection are required. 11.3 million women in the USA had at least one UTI in 1995 and 25–40% of women in the USA age 20–40 years have had a UTI. Some estimate that UTIs cost at least 1 billion US dollars

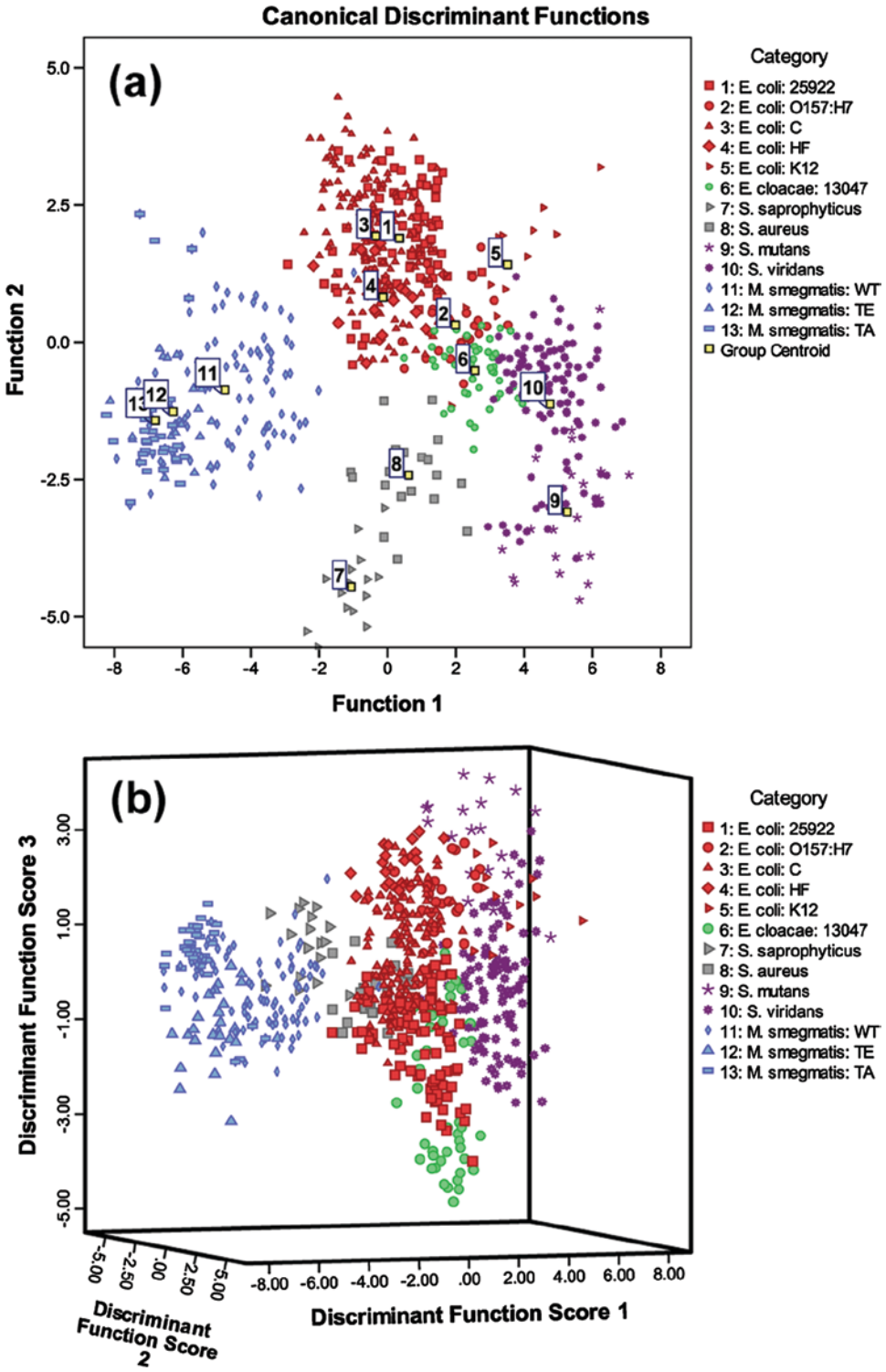

Figure 4. The results of a discriminant function analysis performed on over 600 spectra from 13 specimens of bacteria. The 13 specimens were comprised of bacteria representing five genera. Included in the analysis were two species of *Staphylococcus* (*aureus* and *saprophyticus*) in grey, two species of *Streptococcus* (*mutans* and *viridans*) in purple, five strains of *E. coli* in red, one strain of *Enterobacter cloacae* in green, and three strains of *Mycobacterium smegmatis* in blue. (a) Only the first two of 12 canonical discriminant functions are shown, as well as the 'group centroids' or centres of mass of the clusters of points. (b) The first three canonical discriminant functions are shown in a corresponding 3D plot and the group centroids are removed for clarity.

per year and the overall cost of prescriptions alone to treat UTIs in 1995 was more than $218 million.

An EMMA benchtop apparatus designed to identify bacteria in a generic fluid sample has numerous applications outside of clinical laboratories. It could be used to monitor water for environmental assays (well water or swimming beaches) or for public-consumption. It could be used to monitor food/beverage preparation and packaging in a rapid non-culture

approach. As of 2000, 76 million foodborne illnesses occurred each year in the USA and accounted for 325,000 hospitalizations and 5000 deaths [73]. The US Department of Agriculture (USDA) estimates $2.9–$6.7 billion will be lost annually due to medical costs and lost productivity caused by major foodborne illnesses [74]. It is worth noting that the detection and identification of foodborne pathogens in this sector continue to rely on conventional culturing techniques. Any situation that involves the detection and identification of bacteria in a liquid sample could potentially benefit from the EMMA technology whose attributes include (1) rapid analysis (minutes), (2) high-sensitivity, (3) minimal sample preparation, (4) reasonable cost, and (5) ease of use.

4.2. Surface/air contamination

Apart from liquid samples, EMMA could be used to detect bacterial surface contamination. Hospital surfaces could be instantly monitored via swipe protocols that have already been proven effective for explosive detection and identification. Sampling may be done on the surface of the swipe, or it may be rinsed or washed in a buffered solution and the resulting liquid analysed using the same kind of benchtop monitor described earlier. To address this need, a commercial instrument for analysing residues sampled via disposable surface swabs in real-time has recently been developed by Applied Photonics (Skipton, North Yorkshire, UK).

Recent work on UV laser decontamination is suggestive that a single LIBS device can actually perform multiple steps in a surface decontamination procedure including (1) determining the location of contamination (this could be done in a stand-off mode); (2) decontaminating the surface via a rastered UV photon laser scan; and (3) verifying that the decontamination has been successful by subsequent testing. This work builds on the current investment in LIBS technology to detect and identify surface trace residues of explosives on a wide variety of surfaces.

Air monitoring/decontamination could be facilitated by the EMMA technology, as it has been shown previously that single bioaerosal particles can be analysed in an 'online' gas flow geometry [50]. Bioaerosals can also be monitored via SIBS or 'spark-induced breakdown spectroscopy'. The SIBS system is a component of the Rapid Agent Aerosol Detector (RAAD) which, in turn, is a part of the Joint Biological Point Detection System (JBPDS).

4.3. Multi-drug resistant pathogens/MRSA

Efforts to track, contain, and control antibiotic resistant pathogens are sure to dominate efforts and activities in health care systems in the next decade. Multi-drug resistant (MDR) strains of bacteria are an increasing medical concern, as are the methicillin-resistant *Staphylococcus aureus* (MRSA) and the ever-increasingly worrisome 'super-bugs'. MRSA, which was initially reported among injecting drug users in Detroit in 1981 [75], has now become the most frequent cause of skin and soft tissue infections presenting to emergency departments in the USA [76]. In a hospital environment, MRSA infections are associated with greater lengths of stay, an increased rate of mortality, and significantly increased expenses compared to patients with no infections and even patients with methicillin-susceptible staphylococci infections [77,78]. Specifically, hospital-acquired MRSA infections are associated with greater lengths of stay and an increased risk of mortality [77]. For example, for surgical site infections, MRSA was associated with a >12-fold increase in the 90-day postoperative mortality rate [79]. Associated with this increase in mortality is a corresponding increase in patient costs. A conservative estimate is $4000 per patient above and beyond what a treatable non-resistant *Staphylococcus* infection would cost [80]. MRSA infections therefore cost the USA approximately $760 million per year more than methicillin-susceptible *Staphylococci* infections. We estimate that early identification of the MRSA infection (with a LIBS-based diagnostic or other technologies) could save approximately half of that cost, or $380 million per year.

One reason for the expense and the increase in deleterious effects is the difficulty in identifying MRSA infections. Studies have suggested that clinical cultures identify only 15% of patients colonized with MRSA [78], and that up to 90% of patients with MRSA are asymptomatic carriers who are a reservoir for further transmission [81]. It has been shown, however, that early effective screening is the best and only method for reducing rates of MRSA infection [82]. Currently an inexpensive, rapid screening test does not exist. In particular, molecular techniques such as 'rapid' PCR have proven woefully inadequate, costing more than conventional culture methods, and yielding an unacceptably low accuracy rate of only 65% in one study [83]. In addition it was found that these molecular tests were not only more costly than detection by culture, but the specimen processing workload and the need for highly-skilled personnel to perform the test precluded that availability of the test 24 hours a day, seven days a week—an unacceptable lapse in screening coverage [84].

The EMMA technique, with its emphasis on speed, could provide a near real-time diagnostic test obtainable by a non-expert for the detection of antibiotic resistant bacteria. We have previously shown that EMMA can discriminate strains of *E. coli*, specifically live cells of a pathogenic EHEC strain from non-pathogenic strains [66,67], and recently Multari *et al.* [85] have shown that LIBS can be used to distinguish between clonal methicillin-resistant strains of lyophilized *S. aureus*. In addition to this demonstrated specificity, we have observed that EMMA identification is independent of whether the bacterial cells are alive and reproducing, inactivated by bactericidal UV irradiation, or killed by autoclaving [70]. This suggests that samples could not only be tested quickly, but they could be handled safely by clinical staff as well, reducing cost and biohazard risk to personnel.

5. Advanced approaches of EMMA

It is possible that future LIBS-based biodetection technologies may not rely solely on the pathogen's unique atomic composition for identification. While LIBS has shown itself to be useful for bacterial identification, its utility could perhaps be improved by integrating it with other advanced approaches

that would make the technique even more flexible and powerful.

5.1. LIBS-based immunoassay tags

One example of this is the idea of using immuno-assay tags in conjunction with LIBS. In this idea, unique elemental nanoprobes are prepared and biochemically integrated into pathogen-specific immunoassay probes which are then used in a manner similar to fluorescently labeled antibody tags [86]. The elements used in these 'tag' nanoprobes could be unique to each pathogen of interest, uncommon enough that they would not normally be observed in any LIBS plasma, and can be used in combination with each other. For example, combining 11 different elements (i.e. iron, gold, silver, platinum, aluminium, titanium, vanadium, nickel, zinc, tin and copper) gives more then 1000 types of composite particles. Melikechi and Markushin at Delaware State University already have a patent pending for these 'mono- and multi-element coded LIBS assays [87]'. Each of these composites would be unique in elemental content and could potentially be used as a microtag for detecting and identifying pathogens in a multi-element coded LIBS assay.

Just as molecular fluorescence tags are commonly used, these elemental nanoprobes would be immuno-conjugated to create pathogen-specific biomarkers containing a known element or mixture of elements. These biomarkers would be introduced into the suspect bacteria-containing specimen, dispersed, and the sample would be collected, washed, and then analysed via LIBS. If no pathogens are present, no elemental signal would be registered. The presence of a specific atomic emission in the LIBS signal would indicate the presence of a specific pathogen. This has already been used to detect a model protein at the 30 ppb level using silicon as the elemental nanoprobe material [86].

The advantage that this technique has over fluorescently labelled tags (which will almost certainly be more sensitive) is the ability to form combinations and to identify multiple species of bacteria in extremely 'mixed' samples. The molecular basis for fluorescent tags predicates that only a limited number of fluorescent colours are accessible. Thus only a limited number of different types of bacteria can be detected at one time. Usually fluorescent probes utilize a single fluorescent dye resulting in specificity (yes–no) for only a single organism. This is clearly not useful for screening clinical or environmental samples which may contain a wide variety of bacteria.

5.2. Sensor fusion

Because LIBS is an all-optical technique, it naturally lends itself to a 'fusion' with other optical sensing modalities. The most obvious of these is Raman (molecular) spectroscopy. An integrated LIBS-Raman sensor fusion device has the potential to identify a pathogen on the basis of its elemental and molecular composition, increasing the specificity above that attainable by either on its own. Hybrid LIBS-Raman measurements have been performed over the last decade, particularly with regards to demonstrating its feasibility for stand-off or remote analysis [88,89]. More recently, sensor fusion utilizing a single device has been demonstrated by Wiens *et al.* [90] for the chemical analysis of rock, soils and materials of geological interest for planetary explorations and space missions and also by Laserna *et al.* [91] who demonstrated true real-time molecular and multi-elemental stand-off analysis of explosives utilizing a single laser event at distances up to 20 m.

To date little published work has explored the combination of these two complementary techniques for biomedical applications, such as pathogen identification and tissue classification [92]. Some commercial interest exists in this area of study (i.e. Chemimage Corp., Pittsburg, PA, USA) particularly for rapid reagentless biodetection for military and security applications.

6. Future biomedical applications of LIBS

As already mentioned, one of the advantages of the reagentless LIBS technology is its ability to perform in a stand-off or remote configuration. Remote operation removes the operator from exposure to the material being analysed or the environment in which the material is located, increasing operator safety. This is particularly important in battlefield environments, where it may not be possible for a human to safely reach the injured soldier, the soldier may be in a remote environment inaccessible to medical personnel, or where the soldier may have been exposed to unknown hazards which require remote diagnostics prior to human contact. This increases the significance of this technology to the military and other hostile environments/localities where 'extreme' medicine may need to be practiced (remote bases, Antarctic facilities, submarines, drilling/mining facilities, etc).

6.1. LIBS and exobiology

Another robotic application of LIBS is its use on the Mars Science Laboratory (successfully launched in November 2011) as part of the ChemCam instrument package which will use LIBS to quickly and accurately determine elemental compositions of surface materials up to seven metres away from the rover. The same chemometric algorithms that are being designed to identify inorganic surface materials can be utilized to identify signs of organic material, evidence of potentially habitable environments at some point in the past, if different reference libraries are constructed. The ChemCam team is currently preparing for this application [93]. While it is generally assumed that LIBS is appropriate only for inorganic (i.e. rock) identification, we have shown that its ability to identify and discriminate biological targets may render it useful for searches for signs of exobiology. It remains to be seen how biological identification and discrimination is affected by the various atmospheres that would be encountered in exobiological applications. Plans are underway to simulate these tests in terrestrial cave robotic excursions.

6.2. Advances in the LIBS technology

The LIBS technology is presently undergoing significant advances both in the components (lasers, spectrometers, optical delivery systems) as well as in advanced data processing. New laser devices are being developed to be smaller, lighter and less expensive than the current systems. Even the

Table 1. Demonstrated LIBS capabilities with relevance to medical applications and specific potential future medical applications.

Demonstrated LIBS capability….	…can lead to this capability….	…which could be applied in these medical applications
delivery of laser pulses & collection of plasma emission through optical fibre	LIBS *in vivo* analysis of tissue for real-time analysis	*in vivo* or *in vitro* "optical biopsies" (discrimination of cancerous / malignant / pre-cancerous tissues)
spectra obtained underwater (via fibre coupling) and in high-pressure environments		*in vivo* identification of ulcerated tissue
differentiation of malignant / healthy tissues & classification of different malignancies		*in vivo* stone analysis
elemental analysis of calcified tissues ("stones")		real-time (during procedure) identification of dental caries tissue
elemental analysis of bone/tooth tissue; discrimination of dental caries from healthy tissue		*in vivo* measurement of heavy metal concentrations in tissues with high-spatial resolution (i.e. in different parts of bone, in joints, in different regions of liver, etc.)
sensitivity to all heavy metals (e.g. lead, chromium) and sensitive detection of metals in human tissue and surrogates		
rapid bacterial identification based on elemental composition	real-time diagnosis of pathogen presence in human fluids (blood, urine, CSF, sputum)	autonomous (no expertise required) identification of bacteria in human fluid specimen
rapid discrimination of closely-related bacterial strains		rapid screening for MRSA infections in hospital
enhancement of specificity/sensitivity using LIBS/Raman fusion		real-time meningitis test
enhancement of LIBS specificity by multi-element tagging of macromolecules		rapid strain-classification for epidemic control in hospitals/other
		on-line sensing of water for purity/contamination monitoring
		screening of asymptomatic persons via swab or saliva contribution for early infection detection (e.g. airport screening)
		monitoring of surface contamination for hygiene compliance
		office based UTI test
		remote operation (i.e. on a medical robot) for real-time patient analysis in hostile / battlefield environments

currently used lasers, most notably the Nd:YAG flashlamp pumped systems, are very reliable and robust. The spectrometers, particularly the CCD-based systems, are also very stable and robust. There are no anticipated 'showstoppers' with regards to developing medical devices for both the laboratory/clinic as well as for use in the field, which is always a greater challenge due to size, weight and power constraints. As specific measurement needs and applications are defined, the LIBS devices will be developed for optimal performance with regards to sample introduction, speed of analysis, and ease of use by non-experts.

7. The future of LIBS in the biomedical world

This is an exciting times for bio-LIBS research. Preliminary experiments have been conducted in a variety of biomedical and medical fields and highly encouraging and intriguing results have been obtained. What is required now is a significant investment by various entities (federal funding agencies, private corporations, etc) to apply LIBS to a small subset of specific biomedical problems with the end goal of developing a commercial instrument designed to address this problem for non-scientific end-users. This will require extensive sample handling/preparation experiments to establish rigorous but highly reproducible testing protocols and procedures. Eventually extensive clinical blind trials will need to be conducted with the results confirmed by established 'gold-standard' methods. The clinical specificity and sensitivity will need to be determined through an analysis of the results of blind trials.

Nonetheless, all previous results to date indicate that this effort could lead to an instrument that would have a tremendous impact on the specific biomedical application under investigation. In table 1, we summarize the specific technical demonstrations that have already appeared in peer-reviewed literature and we extrapolate how these abilities may be applied to important clinically relevant problems. By bringing the speed, power, portability and flexibility of the LIBS technique to the biomedical marketplace, we have the ability to open a completely new avenue in medical science in the 21st century.

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