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Discrimination of soft tissues using laser-induced breakdown spectroscopy in combination with k nearest neighbors (kNN) and support vector machine (SVM) classifiers

Xiaohui Li *, Sibo Yang, Rongwei Fan, Xin Yu, Deying Chen

National Key Laboratory of Science and Technology on Tunable Laser, Harbin Institute of Technology, Harbin 150080, China
Institute of Opto-electronics, Harbin Institute of Technology, Harbin 150080, China

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ABSTRACT

In this paper, discrimination of soft tissues using laser-induced breakdown spectroscopy (LIBS) in combination with multivariate statistical methods is presented. Fresh pork fat, skin, ham, loin and tenderloin muscle tissues are manually cut into slices and ablated using a 1064 nm pulsed Nd:YAG laser. Discrimination analyses between fat, skin and muscle tissues, and further between highly similar ham, loin and tenderloin muscle tissues, are performed based on the LIBS spectra in combination with multivariate statistical methods, including principal component analysis (PCA), k nearest neighbors (kNN) classification, and support vector machine (SVM) classification. Performances of the discrimination models, including accuracy, sensitivity and specificity, are evaluated using 10-fold cross validation. The classification models are optimized to achieve best discrimination performances. The fat, skin and muscle tissues can be definitely discriminated using both kNN and SVM classifiers, with accuracy of over 99.83%, sensitivity of over 0.995 and specificity of over 0.998. The highly similar ham, loin and tenderloin muscle tissues can also be discriminated with acceptable performances. The best performances are achieved with SVM classifier using Gaussian kernel function, with accuracy of 76.84%, sensitivity of over 0.742 and specificity of over 0.869. The results show that the LIBS technique assisted with multivariate statistical methods could be a powerful tool for online discrimination of soft tissues, even for tissues of high similarity, such as muscles from different parts of the animal body. This technique could be used for discrimination of tissues suffering minor clinical changes, thus may advance the diagnosis of early lesions and abnormalities.

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1. Introduction

Laser-induced breakdown spectroscopy (LIBS) is an analytical spectroscopic technique that utilizes the emission of laser-induced plasma generated in or on samples to perform quantitative and qualitative analyses. It has many advantages, including no or less sample preparation, simplicity in experimental apparatus, capability of detection of almost all the elements, and feasibility of long-distance and handheld on-site detections [1]. With such merits, LIBS has been applied to various gaseous, liquid and solid samples in different areas, including pollution control [2–4], hazardous material detection [5], agricultural and soil science [6–9], food science [10], archeology [11], geography [12,13], online sorting and quality control [14], deep-space and oceanic exploration [15–17], and biomedical applications [18–20].

LIBS has been actively applied in biomedical area in recent years. It has been used for analysis and discrimination of micro bioaerosols (bacteria, fungi, spores, molds, pollens, proteins, etc.) [21–23], bioliquids (blood and urine) [24–26], calcified tissues (teeth, bones, etc.) [19,27,28] and soft tissues [29–35]. For soft tissues, LIBS has been used for diagnosis of malignancies [29,32,35] and classification of chicken, pork and beef tissues [31,33,34,36]. Due to the intrinsic softness and heterogeneity of the tissues, most of the soft tissue samples were pretreated to improve the performances of LIBS analysis, for example, by preparing the tissues into histological sections after paraffin imbedding, freezing the tissues under low temperature conditions [29,34], or pressing the tissues into pellets [35]. However, in certain applications, such as online identification of abnormalities and malignancies or real-time monitoring during laser surgery process, the tissues cannot be pretreated and thus the LIBS measurements should be performed

* Corresponding author at: Institute of Opto-electronics, Harbin Institute of Technology, Room 204, Building 2A, 2 Yikuang Street, Harbin 150080, China.
E-mail address: lixiaohui@hit.edu.cn (X. Li).

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directly on the soft tissues under the open air condition. Currently, only very limited work has been done on discrimination of *"real"* soft tissues using LIBS [30,31,33]. Kanawade et al. investigated classification of pork fat, muscle, nerve and skin using LIBS to provide an online feedback mechanism for laser surgery [30,31]. Statistical methods such as principal component analysis (PCA) and linear discriminant analysis (LDA) [31], or intensity ratios of atomic lines [30] were used for classification of the tissues. Mehari et al. investigated differentiation of *ex vivo* pork nerve and fat tissues using LIBS in combination with PCA and intensity ratios of atomic lines [33]. Furthermore, in some medical cases, such as diagnosis of early lesions and abnormalities, it is expected to distinguish soft tissues suffering only minor clinical changes, thus the potential of LIBS for discrimination of soft tissues with high similarities needs to be investigated.

To distinguish soft tissues of high similarity using LIBS, multivariate statistical methods usually need to be used to improve the discrimination performances. LDA has been commonly used in discrimination analysis based on LIBS spectra [26,31,35,37], however it uses linear treatment, thus cannot deal with the underlying nonlinearity issues, such as self-absorption of the plasma and fluctuation of the sample matrix [38]. With such limitations, LDA may suffer serious problems of robustness and accuracy on substances with highly similar composition. k nearest neighbor (kNN) and support vector machine (SVM) classifiers are two classification methods that can deal with the nonlinearity issues. They have exhibited high robustness in discrimination of complex substances, such as plastics [39,40], pharmaceutical samples [38], and bacillus spores [41], and may also perform well on discrimination of soft tissues.

In this work, discrimination of fresh soft tissues using LIBS in combination with kNN and SVM classifiers has been investigated. Pork fat, skin, and three types of muscle (including ham, loin and tenderloin) tissues are obtained and manually cut into slice samples. A 1064 nm pulsed Nd:YAG laser is used to generate the LIBS spectra on the samples. Discrimination analysis is firstly performed between fat, skin and muscle tissues and then further performed between highly similar ham, loin and tenderloin muscles. Two multivariate statistical methods, i.e., kNN and SVM classifiers, are used for the discrimination analyses. The performances of the discrimination models, including the accuracy, sensitivity and specificity, are evaluated using 10-fold cross validation. The results show that, the LIBS technique in combination with multivariate analyses could serve as an effective discrimination method for soft tissues, even for the tissues with high similarities, such as muscle tissues from different parts of the animal body.

2. Materials and methods

2.1. Soft tissue samples

Five types of soft pork tissues, including fat, skin, ham muscle, loin muscle, and tenderloin muscle, were purchased from the local market. Six different samples were obtained for each type of tissue, therefore, 30 samples in total were obtained. These samples were randomly obtained from different animals to ensure the generality of the results. After numbering, the samples were kept in a 4 °C refrigerator before further processing.

To perform the LIBS measurements, the samples were manually cut into approximately 2 mm thick slices using a scalpel. It should be pointed out, although we have made great effort to achieve a homogeneous and flat sample surface, the sample surface still suffers inhomogeneity and unflatness, which may affect the discrimination performances of LIBS. The tissue slices were washed by sterile saline solution twice to remove the blood spots on the surface, and then by distilled water twice to remove the residual sterile saline solution on the surface. The tissue slices were then wiped with cleaning paper to remove the residual water on the surface.

2.2. LIBS measurements

The LIBS measurements were conducted on the same day of sample collection. The measurements were performed in the open air environment to simulate the real clinical application conditions. The sequence of the samples to conduct the measurements was randomly set. The prepared tissue slice sample was placed on a remotely controlled three-dimensional translation stage (OptoSigma). A Q-switched Nd:YAG laser with wavelength of 1064 nm and pulse width of ~8 ns was used to generate the plasma. The experimental conditions were optimized to obtain high quality LIBS spectra in terms of high signal-to-noise ratio (SNR), high signal-to-background ratio (SBR) and rich emission lines for discrimination analysis. The quality of LIBS spectra is affected by many experimental parameters, including laser fluence, detection delay, integration time and number of accumulations. In practice, we should fix some parameters first, and then optimize other parameters. In this paper, the laser fluence was firstly adjusted to obtain LIBS spectra that were strong enough to take advantage of the dynamic range of the spectrometer. The pulse energy was fixed to ~73 mJ, and the fluctuation of the pulse energy was within 1%. The laser was focused onto the tissue sample with a plane-convex lens with a focal length of 75 mm. The emission of the plasma was collected using a focal lens with a focal length of 50 mm and a diameter of 25 mm, coupled into a 4-in-1 fiber bundle and collected by a four-channel spectrometer (AvaSpec-ULS2048-4, Avantes). The spectrometer covers the spectral range of 210–850 nm with spectral resolution of 0.09–0.22 nm. The delay time of the spectrometer was optimized based on the SNR and SBR of the spectra. The delay time was set to 3 μs following the onset of the plasma. Since the minimum exposure time of the spectrometer was limited to 1.05 ms, the detection gate was fixed to 5 ms to collect the emission during the whole plasma lifetime after the set delay time. To avoid laser drilling on the soft sample, the sample was translated following a zigzag route relative to the plasma with a step size of 200 μm. After moving a step, the driver (SHOT-304GS, OptoSigma) of the translation stage will generate an external trigger pulse for a digital delay generator (DG645, Stanford Research Systems), which then provides external triggers for the laser and the spectrometer. After firing the laser, the translation stage then moves the next step, such that the laser ablates each spot on the sample only once. The LIBS spectra were averaged 25 times to mitigate the effect of fluctuations of the sample matrix and the ablation process. For each tissue sample, 100 averaged spectra were collected. Therefore, 3000 spectra in total were obtained for the following discrimination analysis.

2.3. Data analysis

For the discrimination applications using LIBS, the spectra are usually normalized to offset the spectral fluctuations caused by the variation of laser pulse energy and matrix conditions. The normalization is usually performed relative to a specific emission line [29,31,42]. In this work, the Na I 588.99 nm line was selected for normalization because of its good SNR and comparable values among the three types of highly similar muscle tissues. After normalization, several representative emissions were selected for the tissue discrimination. Two discrimination analyses were performed in this work. In the first case, discrimination between the fat, skin and muscle tissues was investigated. All the 30 tissue samples were used for the discrimination analysis. Here, the ham, loin, and tenderloin muscles were all treated as the "muscle" class. In

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