Spectrochimica Acta Part B 139 (2018) 63–69

Contents lists available at ScienceDirect

Spectrochimica Acta Part B

journal homepage: www.elsevier.com/locate/sab

Diagnosis of human malignancies using laser-induced breakdown spectroscopy in combination with chemometric methods

Xue Chen [a,*,1], Xiaohui Li [b,c,**,1], Xin Yu [b,c], Deying Chen [b,c], Aichun Liu [a]

[a] Department of Hematology, Harbin Medical University Cancer Hospital, Harbin 150081, China
[b] National Key Laboratory of Science and Technology on Tunable Laser, Harbin Institute of Technology, Harbin 150080, China
[c] Institute of Opto-electronics, Harbin Institute of Technology, Harbin 150080, China

ARTICLE INFO

Article history:
Received 1 September 2017
Received in revised form 25 October 2017
Accepted 27 November 2017
Available online xxxx

Keywords:
Cancer diagnosis
Laser-induced breakdown spectroscopy
Serum
Discriminant analysis
k nearest neighbor

ABSTRACT

Diagnosis of malignancies is a challenging clinical issue. In this work, we present quick and robust diagnosis and discrimination of lymphoma and multiple myeloma (MM) using laser-induced breakdown spectroscopy (LIBS) conducted on human serum samples, in combination with chemometric methods. The serum samples collected from lymphoma and MM cancer patients and healthy controls were deposited on filter papers and ablated with a pulsed 1064 nm Nd:YAG laser. 24 atomic lines of Ca, Na, K, H, O, and N were selected for malignancy diagnosis. Principal component analysis (PCA), linear discriminant analysis (LDA), quadratic discriminant analysis (QDA), and k nearest neighbors (kNN) classification were applied to build the malignancy diagnosis and discrimination models. The performances of the models were evaluated using 10-fold cross validation. The discrimination accuracy, confusion matrix and receiver operating characteristic (ROC) curves were obtained. The values of area under the ROC curve (AUC), sensitivity and specificity at the cut-points were determined. The kNN model exhibits the best performances with overall discrimination accuracy of 96.0%. Distinct discrimination between malignancies and healthy controls has been achieved with AUC, sensitivity and specificity for healthy controls all approaching 1. For lymphoma, the best discrimination performance values are AUC = 0.990, sensitivity = 0.970 and specificity = 0.956. For MM, the corresponding values are AUC = 0.986, sensitivity = 0.892 and specificity = 0.994. The results show that the serum-LIBS technique can serve as a quick, less invasive and robust method for diagnosis and discrimination of human malignancies.

1. Introduction

Despite continuous research efforts, cancer remains a lethal threat affecting millions of people around the world. According to the statistics released by World Health Organization, malignant neoplasms are ranked as the second leading cause of deaths in the world. In 2015, malignant neoplasms caused more than 8.7 million deaths around the world [1]. Malignant neoplasms are also the second leading cause of deaths in China. In 2015, more than 2.3 million people died from malignant neoplasms in China [1]. One import reason for the high mortality of malignancies is that most malignancies are difficult to diagnose at the early stages, such that when diagnosed, the malignancies have already developed into advanced stages and the best opportunities for treatment have missed. Therefore, early diagnosis of the malignances is very important for effective treatment and control of the progression of cancers, and may help to reduce the morbidity and mortality of cancers.

Lymphoma and multiple myeloma (MM) are two major cancers affecting the lymphatic and hematopoietic systems. They accounted for about 3.2% and 0.8% respectively of the total new cancer cases, and 2.7% and 1% respectively of the cancer deaths worldwide in 2012 [2]. In China, about 29,300 and 9200 people died from lymphoma and MM respectively in 2015 [1]. Despite the progresses made in the last years, detection of lymphoma and MM remains a significant clinical challenge. Because most cases with the two malignancies typically develop with no specific symptoms, the majority of the cases are discovered at the advanced stages, which are usually related to bad prognosis. Therefore, new technical measures need to be developed to realize early diagnosis of these malignancies.

One promising technique for cancer diagnosis and discrimination is laser-induced breakdown spectroscopy (LIBS). It has many merits to be easily adopted for clinical applications, including simplicity in apparatus, no or less sample preparation and possibility of stand-off operations

* Correspondence to: X. Chen, Department of Hematology, Harbin Medical University Cancer Hospital, 150 Haping Road, Harbin 150081, China.
** Correspondence to: X. Li, Institute of Opto-electronics, Harbin Institute of Technology, Room 204, Building 2A, 2 Yikuang Street, Harbin 150080, China.
E-mail addresses: chenxuecherry@163.com (X. Chen), lixiaohui@hit.edu.cn (X. Li).
[1] Contributed equally as co-first authors and co-corresponding authors.

https://doi.org/10.1016/j.sab.2017.11.016
0584-8547/

[3,4]. By generating a plasma directly on the tissue (usually by ablation) and analyzing the LIBS spectra of the plasma, the malignancies can be discriminated from the normal tissues [5–7]. The discrimination was made by direct comparison of the intensities or intensity ratios of specific atomic lines of malignant and normal tissues [5,6], or by further analysis in combination with chemometric methods [7]. For example, Kumar et al. proposed discrimination of hemangiosarcoma and normal dog liver cells using intensity ratios of Ca, Cu, and Na with K [5]. El-Hussein et al. demonstrated identification and classification of breast and colorectal cancers via detection of the abundance of Ca and Mg in the malignant tissues [6]. Han et al. realized discrimination of melanoma from surrounding dermis on a mice model by analyzing the quantitative difference of Ca and Mg in the tissues and by using principal component analysis and linear discriminant analysis (LDA) [7]. However, it should be noted that in the aforementioned direct tissue-ablation scheme [5–7], the LIBS acts more as a malignancy *discrimination* method rather than a *diagnosis* method, because before applying the LIBS discrimination, the malignancies need to be pre-located using other techniques. An alternative approach for malignancy diagnosis using LIBS is detection of cancer-associated biomedical markers or signatures in the biological fluids, such as urine, blood, etc. For example, Markushin et al. reported detection of cancer antigen 125 in blood serum using microparticle-tagged LIBS [8]. Recently, Melikechi et al. reported age-specific discrimination of blood plasma samples of healthy and ovarian cancer prone mice using LIBS and LDA and random forest analysis [9], which indicated the feasibility of malignancy *diagnosis* using LIBS. However, to our knowledge, diagnosis of human malignancies using LIBS conducted on serum samples of patients baring the malignancies has not been reported before.

In this work, we present diagnosis and discrimination of lymphoma and MM using LIBS conducted on serum samples in combination with chemometric methods. The serum samples collected from lymphoma and MM cancer patients and healthy controls were deposited on filter papers and ablated with a pulsed 1064 nm Nd:YAG laser. The LIBS spectra of the serum samples were analyzed using principal component analysis, linear discriminant analysis, quadratic discriminant analysis, and k nearest neighbor classification, to diagnose and discriminate the malignancies. Cross validation was used to evaluate the discrimination models. High discrimination performances in terms of discrimination accuracy, sensitivity and specificity were achieved, indicating that this technique can serve as a quick and robust diagnosis and discrimination method for human malignancies.

2. Materials and methods

The serum samples were collected from the lymphoma and MM patients registered in Department of Hematology, Harbin Medical University Cancer Hospital (HMUCH), and from healthy donators. In total, 48 serum samples from 15 lymphoma patients, 16 MM patients and 17 healthy controls were collected. The cancer patients have been diagnosed using the conventional diagnostics, including laboratory blood and urine tests, bone marrow biopsy, and histological pathology. For the lymphoma patients, the extent of the disease was assessed with the four-stage Cotswolds modification of the Ann Arbor staging system. The numbers of patients at Stage I, Stage II, Stage III and Stage IV are three, four, seven, and one, respectively. Five of them were first diagnosed and had not received any chemotherapy medication when the serum samples were collected. The other ten lymphoma patients were under chemotherapy when the serum samples were collected. For the MM patients, the extent of the disease was assessed with the International Staging System (ISS). Six, one and nine patient(s) were (was) at the ISS I, ISS II and ISS III stages, respectively. All the MM patients were under chemotherapy when the serum samples were collected. All the subjects involved in this work signed informed consent in compliance with Declaration of Helsinki. This clinical procedure was approved by the Clinical Research Ethics Committee of HMUCH. For each subject, about 2.5 mL blood sample was drawn from the vein on the inner portion of the arm near the elbow. Following collection, the blood sample was centrifugalized for 5 min to compact cells. The serum sample was then taken from the upper part and transferred to sterile micro centrifuge tubes. The serum samples were stored at −25 °C until the LIBS measurements. The LIBS measurements were usually performed within 72 h after the sample collection.

The LIBS measurements were conducted on the serum samples following a "first collected, first tested" rule. Thus, the order of measurements could be viewed as random. Before the LIBS measurements, the serum sample was melted at room temperature (~25 °C). Then, 50 μL serum sample was deposited uniformly onto a 2.5 × 1.25 cm^2 quantitative filter paper, a product following Chinese national standard GB/T1914-2007, using a pipettor. The average amount of serum sample on the filter paper was 16 μL/cm^2. According to the standard, the main content of the filter paper is purified cellulose. The ash content of the filter paper is less than 0.01% in weight. From the LIBS spectra, it is shown that little amount of calcium (Ca), sodium (Na) and potassium (K) is present in the ash content. The filter paper was then naturally dried for 20 min to remove the liquid content in the serum sample. With these procedures, the liquid serum samples were converted to solid state to facilitate the measurements. To minimize the contamination from the environment, these steps were performed in an air-filtered laminar flow cabinet.

The dried filter paper was fixed to a programmable three-dimensional translation stage (OptoSigma). A 1064 nm, ~8 ns pulse width, Q-switched Nd:YAG laser was used to generate the plasma. The laser pulse energy was ~73 mJ. The fluctuation of the laser pulse energy was within 1%. The laser was focused onto the filter paper with an $f = 75$ mm plane-convex lens. The emission of the plasma was coupled into a 4-in-1 fiber bundle using an $f = 50$ mm focal lens and transmitted to a four-channel spectrometer (AvaSpec-ULS2048-4, Avantes). The spectrometer covers the spectral range 200–850 nm, with spectral resolution of 0.09–0.22 nm depending on the wavelength. During the ablation process, the filter paper was translated following a zigzag route relative to the plasma with step size of 200 μm. The experimental system was synchronized to ensure a fresh ablation site for each laser pulse. The detection delay of the spectrometer relative to the onset of the plasma was fixed to 5 μs. The delay time was optimized to achieve good signal-to-noise ratio (SNR) and signal-to-background ratio (SBR) for the LIBS spectra. The exposure time of the spectrometer was fixed to 2 ms, so the emission of the whole plasma lifetime after the delay time was collected. For each serum sample, 100 LIBS spectra were collected, and each LIBS spectrum was obtained by averaging 25 independent spectra. With the laser working at 5 Hz, the typical time to conduct the measurements for each sample was 8.33 min (collection of 2500 independent spectra). The ablation area for each sample was about 1 cm^2, thus only 16 μL serum sample was consumed for each test. In total, 4800 LIBS spectra were collected for further analysis.

The experimental parameters were carefully maintained to be consistent during the measurements, including the sample preparation, detection delay and optical alignment. The reproducibility of the LIBS spectra was estimated by measuring the fluctuation of the shot-to-shot intensity ratios of Na D lines (588.99 nm and 589.59 nm) of the 25-time averaged spectrum data sets [9]. The reproducibility of the 100 spectra of each sample is less than 1% and the corresponding value across all the samples is about 6%.

3. Data analysis

Shown in Fig. 1 are the LIBS spectra of a serum sample of a healthy control and an empty filter paper in the spectral region of 350–425 nm. The spectra are normalized to the CN B-X $\Delta v = 0$ band head at 388.34 nm [10]. It can be seen that, after normalization, the two spectra have almost the same CN emission profile. This indicates that the CN emission is mainly from the filter paper. The CN emission is stable and is